Biochemical fuel cells

Murray, K. D.

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BIOCHEMICAL FUEL CELLS

submitted by

K.D. MURRAY

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ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

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SUMMARY

The project involved a study of biochemical fuel cells employing redox enzymes to effect the efficient transduction of chemical into electrical energy. The use of carbon cloth to provide a cheap, high surface area electrode material as an alternative to the precious metals that have been used in previous enzyme fuel cells was examined. Three enzymes were used in the study: alcohol oxidase, yeast alcohol dehydrogenase and glucose oxidase. The alcohol oxidase studied was found to be unsuitable for use in the experimental fuel cell as it underwent a substrate independent reaction with the mediator. Yeast alcohol dehydrogenase was also unsuitable as it was unstable in the fuel cell; immobilisation while enhancing the stability did not produce a satisfactory solution.

Glucose oxidase appeared to be well suited for use in the fuel cell. Of the mediators tested, hydroxyethylferrocene gave the most effective electron transfer in the anode. The novel application of hydroxyethylferrocene made it possible for the anodic compartment to function under aerobic conditions. Using this system, glucose was converted to gluconic acid and electricity at a coulombic efficiency >90%.

The stability of the fuel cell output was not dependent on the adsorption of enzyme or mediator to the anode, but on the pH and the cathodic reaction. This prompted an investigation of the cathodic reaction, a subject which has previously received little attention.

It was found that for high currents the reduction of oxygen at the cathode required a low pH, which proved difficult to maintain. A number of alternative cathodic reactions were examined, and the reduction of potassium ferricyanide was found to produce a high output with increased, but not ideal, stability. The fuel cell produced a current density of $1.0Am^{-2}$ with a power density of $176mWm^{-2}$. Factors affecting the output of the cell included the rate of electron transfer at the anode, the cathodic reaction and the cell design.

ABBREVIATIONS

ABTS	-	2,2' Azinobis (3-ethyl-benzothiazoline-6-sulphonic acid) diammonium salt
ADH	-	Alcohol dehydrogenase
AOD	-	Alcohol oxidase
BQ	-	Benzoquinone
BSA	-	Bovine serum albumin
DCM	-	Dichloromethane
DCPIP	-	Dichlorophenolindophenol
DMF	-	Dimethylformamide
EC	-	Enzyme Commission
FAD	-	Flavin adenine dinucleotide
FMCA	-	Ferrocene monocarboxylic acid
GOD	-	Glucose oxidase
HEF	-	Hydroxyethylferrocene
MB	-	Methylene blue
MV	-	Methyl viologen
NAD	-	Nicotinamide adenine dinucleotide
NHE	-	Normal hydrogen electrode
ox	-	Oxidised species
PES	-	Phenazine ethosulphate
PMS	-	Phenazine methosulphate

Abbreviations (continued)

POD	-	Peroxidase
red	-	Reduced species
SCE	-	Saturated calomel electrode
TMPD	-	Tetramethylphenylenediamine
YADH	-	Yeast alcohol dehydrogenase

Symbols

a	-	Activity
A	-	Specific activity of an enzyme
C	-	Theoretical coulombic yield
e	-	Charge on an electron
E	-	Net reversible potential difference
E_A	-	Potential of anode
E_C	-	Potential of cathode
E°	-	Standard half cell potential
F	-	Faraday constant
G	-	Gibbs free energy
H	-	Enthalpy
l	-	Distance separating two electrodes

Symbols (continued)

ηc	-	Concentration overvoltage
ηo	-	Ohmic overvoltage
ηA	-	Anodic overvoltage
ηC	-	Cathodic overvoltage
$\eta CARNOT$	-	Efficiency of a Carnot cycle transformation
ηE	-	Voltage efficiency
ηF	-	Free energy efficiency
ηI	-	Current efficiency

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1 INTRODUCTION

1.1 FUEL CELLS

1.1.1 Background

A fuel cell, as defined by Wingard et al.(1982), is a device for the direct conversion of chemical energy to electrical energy - without the intermediate conversion into heat and mechanical energy that occurs in conventional forms of electricity production. A fuel cell consists of an anode, a cathode, and a supporting electrolyte medium to connect the two electrodes, and an external circuit to utilize the electricity. Reactants must be supplied to both electrodes as a source for the electron transfer reactions and catalysts are normally present to provide a rapid rate of reaction at each electrode. The thermodynamic efficiency of a fuel cell is simply dependent on the difference in Gibbs free energy between the reactants and the products, with a small loss due to entropy effects. There are no limitations on the efficiency due to the temperature restrictions of the various electromechanical cycles (McDougall, 1976).

The fuel cell concept dates from the beginning of the 19th century and is ascribed to Sir Humphrey Davy (1802), while the possibility of making it a reality was demonstrated by Grove (1839), who obtained a current by supplying hydrogen and oxygen to two platinum electrodes immersed in sulphuric acid. Mond and Langer (1889) continued Grove's work on "gas batteries" and obtained current densities of about 0.2A/cm^2, which compares quite favourably with modern cells. (The current

density is the current obtained per unit surface area of the electrodes). However, the dynamo, a device for converting mechanical work into electrical energy, was developed about this time and the success of this invention caused interest in fuel cells to lapse for almost 60 years.

Until the space exploration programme in the United States, fuel cells were not considered as practical devices for energy transfer mainly due to problems of electrode development, short operational life and high cost. However the development of high pressure hydrogen-oxygen cells for use in Apollo spacecraft brought a rapid advancement in fuel cell technology (Table 1.1).

Table 1.1 Advancements in spacecraft fuel cell performance

Parameter	Performance early 1960s	Performance late 1970s
Operating capability (h)	100	5,000-10,000
Specific cost (US$/kW)	225	3
Specific weight (lb/kW)	89	4

(Wingard et al., 1982)

A diagrammatical representation of a hydrogen-oxygen fuel cell is shown in figure 1.1. Hydrogen is ionised at the anode releasing electrons to the external circuit. The positively charged hydrogen ions are then transported through the electrolyte to the cathode and react with oxygen to form water.

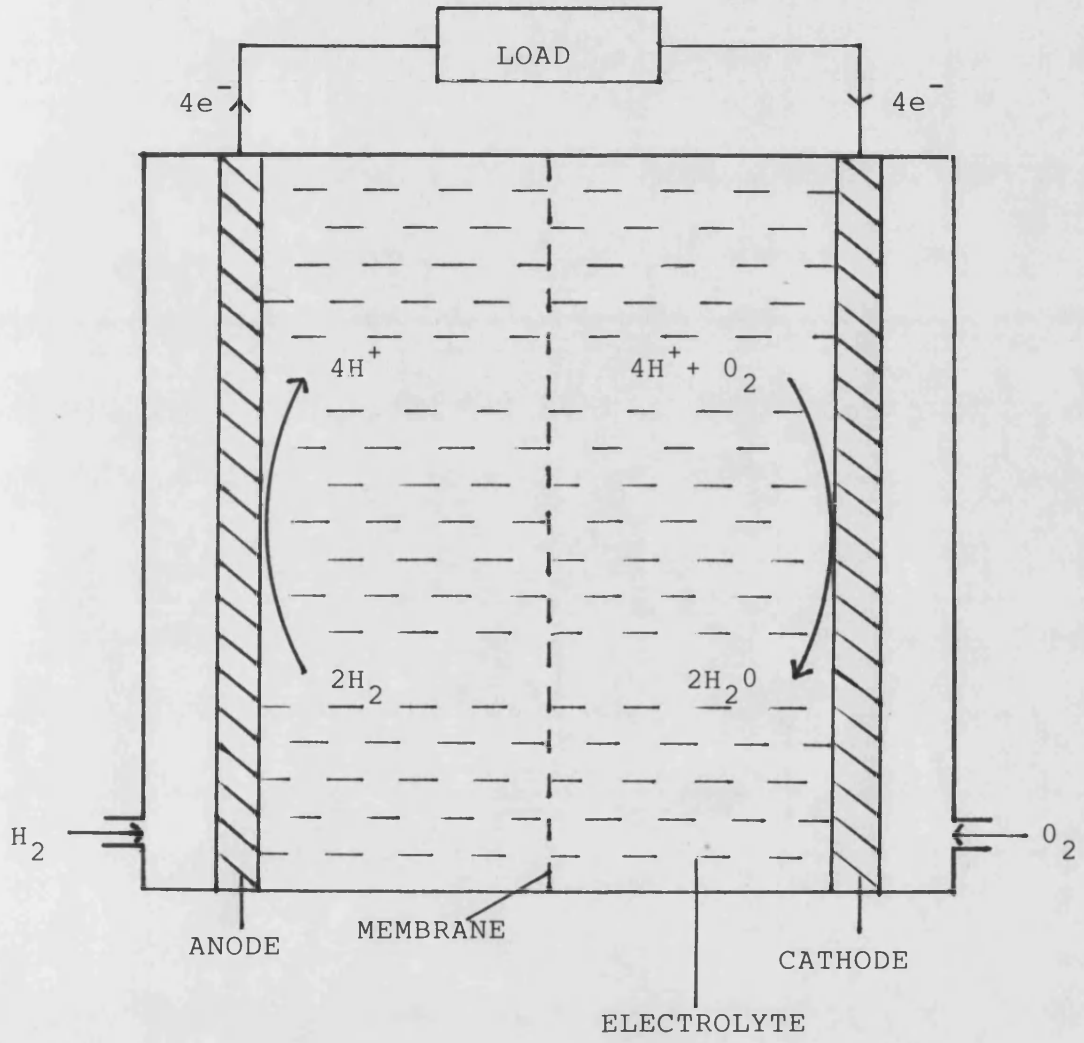


FIGURE 1.1. DIAGRAMMATICAL REPRESENTATION OF A HYDROGEN-OXYGEN FUEL CELL.

1.1.2 Inorganic fuel cells

Inorganic fuel cells can utilise many fuels employing several types of electrolyte. In low temperature fuel cells (<200°C) acidic electrolytes e.g. sulphuric acid, hydrogen bromide and phosphoric acid are most popular, followed by alkaline electrolytes such as potassium hydroxide. Within this temperature range cells with ion-exchange membranes have also been much studied. The membrane is a solid organic or mineral material, saturated with water and consists of a crystalline network with fixed charges. The charges of opposite signs, such as H^+ and OH^- ions, are very mobile and ensure the membrane conductivity. The main advantage of the ion-exchange membrane cell is the possibility of excluding water molecules which result from the reaction at the electrodes. In this way the drawback of the acid or alkaline cell, in which the water formed during operation gradually dilutes the electrolyte and decreases its conductivity, can be avoided (Oniciu, 1976).

Molten salts are excellent ionic conductors and are used as the electrolyte in high temperature cells, the most successful have been mixtures of either lithium and sodium carbonates or lithium, sodium and potassium carbonates operating at temperatures between 400°C and 800°C.

In very high temperature fuel cells (1000°C) ionically conducting solid oxides e.g. ZrO_2 are used as the electrolyte (Smith,1966). The types of material that can be used as catalysts, and for construction

of these inorganic fuel cells are thus severely limited by the temperature and electrolyte requirements.

The fuels utilised in inorganic fuel cells include; (i) hydrogen which will react at any temperature, but is unliquifiable which raises problems of volume in cases where a large quantity is needed in stock, (ii) carbon monoxide and hydrocarbons which are much less reactive than hydrogen but are used successfully to supply the high temperature cells working with molten carbonates, (iii) methanol, which can be used in either an acid or an alkaline electrolyte and, (iv) a range of less common fuels such as hydrazine, sodium amalgam, potassium formate, glycol and glucose.

1.1.3 Applications of fuel cells

Fuel cells have been used in many different fields of activity e.g. as power sources for signal beacons, radio-alarm signals, motor boats, submarines, delivery trucks, domestic use and space capsules (Euler, 1970). Military users have been especially interested in fuel cells due to their tactical advantages of silent functioning, high energy density, long life, simplicity, reliability and high efficiency. Low power systems have been employed for the lighting of field installations and supplying power for detection and transmission units (Verstaete, 1968). High power systems have been developed for light naval craft (McDougall, 1976) and for submarines (Lindström, 1967).

In the Gemini and Apollo spacecraft fuel cells were developed for creating light and powering communications, operation and control, guidance, radar, data storage, and experiments and vehicles on planet surfaces. Fuel cells were again favoured for these applications because of their reliability and high energy density - especially important considering the cost of sending matter into space ($\$2\text{-}10\times10^{3}kg^{-1}$). They also had the added advantage of being able to function in extreme conditions, e.g., weightlessness, absence of an atmosphere, radiation and large temperature fluctuation (Oniciu, 1976).

The many advances in organic fuel cell technology, though, have failed to overcome the major problems of elevated temperature operation, catalyst viability and cost, and electrolyte corrosiveness. The recent development of biochemical (or biofuel) cells, that is, fuel cells in which one or both electrode reactions are promoted or catalysed by biological processes, has led to biologically modified electrode reactions which offer four main advantages, in certain situations, over conventional inorganic catalytic electrodes:-

(i) fast reaction rates at ambient temperatures,

(ii) high substrate specificity,

(iii) high substrate affinity,

(iv) no highly corrosive electrolytes - neutral or nearly neutral aqueous solutions as electrolytes.

The fact that biofuel cells employ a complex protein based system, with certain inherent disadvantages,

means that they will not replace their inorganic predecessors but will considerably extend the range of useful electrochemical processes. Some possible examples are (i) a biofuel cell running on polluted river water, simultaneously purifying the water and producing power, (ii) a biofuel cell powered heart pacemaker fuelled specifically by blood glucose and (iii) an interesting calculation has shown that about 24% of the countries of the world, containing about 37% of the world's population, have a yearly electrical consumption of 20kWh per capita. At a 50% conversion rate, human urinary wastes could supply 18kWh per capita per year (Bockris and Srinivasan, (1969).

In addition it has long been recognised that a biofuel cell may function analytically, the current produced being proportional to the concentration of fuel; the sensitivity and specificity of such configurations has led to much recent commercial interest e.g. biosensors for the measurement of components of body fluids or for use where sensitive fermentation control is needed.

Electron transfer between an enzyme and an electrode also allows the possibility of supplying the reducing equivalents required for many valuable biotransformations directly from an electric current, instead of using expensive cofactors (Turner et al., 1982).

1.2 THEORY

1.2.1 Theoretical efficiency

Figure 1.2 shows a generalised diagram of a fuel cell. Considering the hydrogen-oxygen system, for example, the redox reactions can be written as:-

$H_2 = 2H^+ + 2e^-$ $\quad E^o = 0V$ - anodic

$2H^+ + 2e^- + \frac{1}{2}0_2 = 2H_20$ $\quad E^o = 1.23V$ - cathodic

$H_2 + \frac{1}{2}0_2 = H_20$ $\quad E^o = 1.23V$ - overall reaction

Hence in figure 1.2 the salt bridge would be carrying hydrogen ions from the anode to the cathode. E^o represents the standard half cell potential assuming reversible operation.

The theoretical amount of work that can be obtained from a fuel cell depends on the net release of Gibbs free energy, ΔG, as the reaction proceeds from reactants to products. The free energy is equal to the available work, W_A, and is related to the net reversible potential difference, E (the superscript zero is omitted to indicate that standard conditions are not required), for the anodic and cathodic half cell reaction as given by:-

$$-\Delta G = nFE = W_A$$

where n = number of electrons transferred per mole, and

F = Faraday constant (96,500 coulombs per mole).

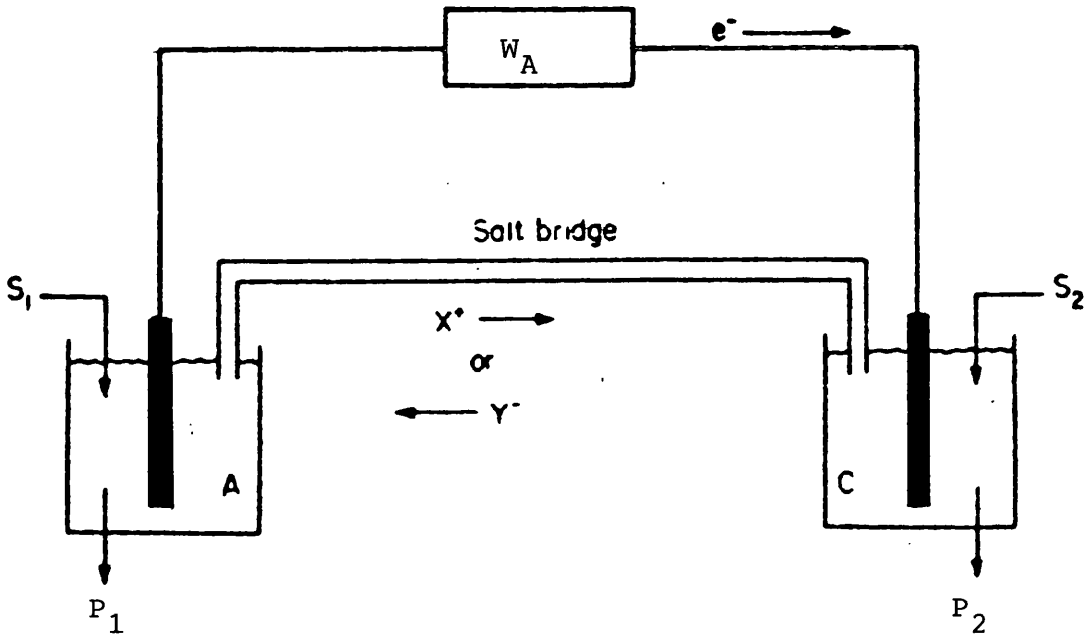


FIGURE 1.2. GENERALISED DIAGRAM OF A FUEL CELL:

A, ANODE; C, CATHODE; S, SUBSTRATES; P, PRODUCTS; X and Y IONIC SPECIES. W_A IS THE LOAD WHERE THE WORK IS PERFORMED. (taken from Wingard et al, 1982)

Combining the definition of Gibbs free energy ($G = H - TS$) and the first law of thermodynamics, then at a constant pressure and temperature, T, the free energy change is related to the change in enthalpy, ΔH, and entropy, ΔS, according to:-

$$\Delta G = \Delta H - T\Delta S$$

The enthalpy decrease ($-\Delta H$) for a chemical reaction can be said to represent the energy put in, and the decrease in Gibbs free energy ($-\Delta G$) can be said to be equal to the maximum electrical energy obtained. Hence the efficiency (η) of a fuel cell can be defined as:-

$$\eta = \frac{\Delta G}{\Delta H} = 1 - \frac{T\Delta S}{\Delta H}$$

For many systems the $T\Delta S$ term amounts to approximately 10% of the enthalpy term, the theoretical efficiency can therefore be as high as 85-90%.

In conventional methods of electricity production, where chemical energy is changed firstly into heat, through burning a fuel, only a part of the amount of heat obtained is convertible into mechanical work, depending on the initial (T_1) and the final (T_2) absolute temperature of the working substance. According to Carnot's theorem the theoretical transformation efficiency is:-

$$\eta \text{ Carnot} = \frac{W}{\Delta H} = \frac{T_1 - T_2}{T_1} = 1 - \frac{T_2}{T_1}$$

The Carnot efficiency increases with T_1 if T_2 remains constant, thus, if T_1 = 1173K (900°C), and T_2 = 373K (100°C) it reaches 70%. In practice the values ascribed to the

main types of thermal engines are much lower e.g. 20-25% for the internal combustion engine, 30-45% for the Diesel engine, and 30-40% for steam turbines (Oniciu, 1976). Many measured fuel cell efficiencies are found to be at 55-60% (Bockris and Reddy, 1970; Williams, 1966).

1.2.2 Overpotentials

The difference between theoretical and measured efficiencies occurs because the theoretical efficiency is obtainable under conditions of no current flow. When the current flows, not all of the potential E^{o} is available to produce work. This is due to overpotential (or overvoltage or polarisation) phenomena which occur at fuel cell electrodes. The overpotential of a particular electrode system is thus defined as the difference between the measured potential under working conditions and the reversible or thermodynamic potential.

Overpotential depends on the load to which the cell is subjected and may have multiple sources, i.e., activation, concentration and ohmic overpotentials. Except for the latter, which usually varies linearly with the current density, the relationship between overpotential and current density is generally complicated, particularly when interaction between different types of overpotentials are also present. However, in many fuel cells in operation, it is possible to identify the type of overpotential prevailing within a given range of current densities - at low current densities activation overpotentials prevail; at medium, ohmic;

and at high current densities, concentration. (Figure 1.3).

The two reaction steps that occur at the electrodes are the cause of activation overpotentials, namely a chemical reaction step and a charge transfer reaction step. The latter is brought about by the movement of electrons across the capacitive or charging layer at the phase boundary between the solid electrode surface and the solution (Bockris and Reddy, 1970). Both these reaction steps have an energy of activation, therefore they can both contribute to the generation of the activation overpotentials. Thus, for a practical fuel cell system it is necessary that these steps proceed at high rates.

The reduction of activation overpotential can be brought about by (i) using a more efficient catalyst to obtain a greater reduction in the activation energy of the chemical reaction, (ii) special treatment of the electrode surface or (iii) employing mediators (see section 1.3.1) to increase the rate of charge transfer.

Concentration overpotentials are caused by slow mass transfer of substrates, charge transfer agents or products towards or away from the electrodes. They can be reduced by thermal or mechanical stirring of the solution, or by employing a rotating electrode.

Ohmic overvoltage (η_{IO}) is due to the resistance set up by the electrolyte solution to the flow of the electric current; in order that the circuit is closed when the fuel cell is on load, electron motion through the external circuit must be associated with ion motion

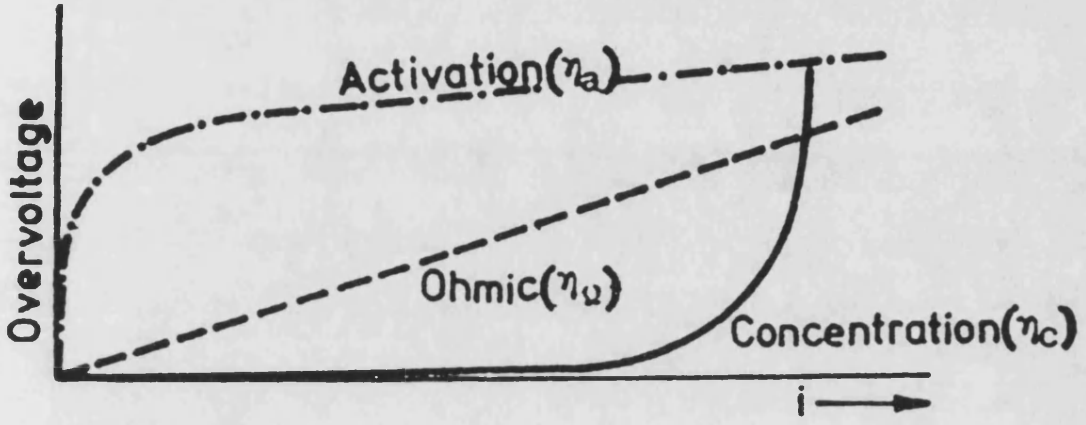


FIGURE 1.3. TYPES OF OVERPOTENTIAL ENCOUNTERED IN FUEL CELLS. (taken from Oniciu, 1976)

within the electrolyte solution. Since the electrolyte has a finite resistance, r, there is a potential drop, η_o, across the cell, which can be calculated according to Ohm's law:

$$\eta_o = Ir$$

The resistance of the electrolyte may be expressed in terms of the distance, l, separating the electrodes of surface area, s, and the conductance of the electrolyte, x:

$$\eta_o = I \frac{l}{sx}$$

Thus during cell construction it is important to minimise the distance between the electrodes. Additional ohmic overpotential may be caused by (i) changes in resistance of the electrolyte because of the removal, production or replacement of ions at the electrodes, (ii) junction potentials that may occur with certain pathways for connecting the two electrode compartments via the electrolyte, and (iii) the resistance of the electrodes themselves with some materials.

A more detailed analysis of the source of overpotentials is not required at this point, but can be obtained from any fuel cell text, e.g., McDougal (1976), Oniciu (1976).

The overvoltage of an individual electrode may be expressed as the sum of contributions from the activation, η_a, concentration, η_c, and ohmic, η_o, overvoltages:

$$\eta = \eta_a + \eta_c + \eta_o$$

For the fuel cell to work, the potential of the cathode, E_C, must be more positive than the potential of the anode, E_A. The active potential, E, available for work is:

$$E = E_C - E_A$$

hence when the cell is under load:

$$E = (E_C - \eta_C) - (E_A - \eta_A)$$

The relationship between the potential available for work and overpotentials as a function of current is shown in figure 1.4.

1.2.3 Voltage Efficiency

One way to assess the effect of overpotentials on a fuel cell's performance is to calculate the voltage efficiency, η_E, which is defined as:

$$\eta_E = \frac{\text{Work done by cell under operating conditions}}{\text{Theoretical work attainable}}$$

the theoretical work attainable = $-\Delta G = nFE$;

the work done by the cell under operating conditions = nFV,

where V = measured potential difference of the cell when operating under an external load.

Hence $\eta_E = \frac{V}{E}$

The net reversible potential difference, E, can be calculated by applying the Nernst equation:

$$E = E^\circ + \frac{RT}{nF} \ln \frac{a_O^x}{a_R^y}$$

where R is the gas constant, T is the absolute temperature, F is the Faraday constant, a is the activity of the species involved, and n is the number of electrons in the reversible equilibrium:-

$$xO + ne^- \rightleftharpoons yR$$

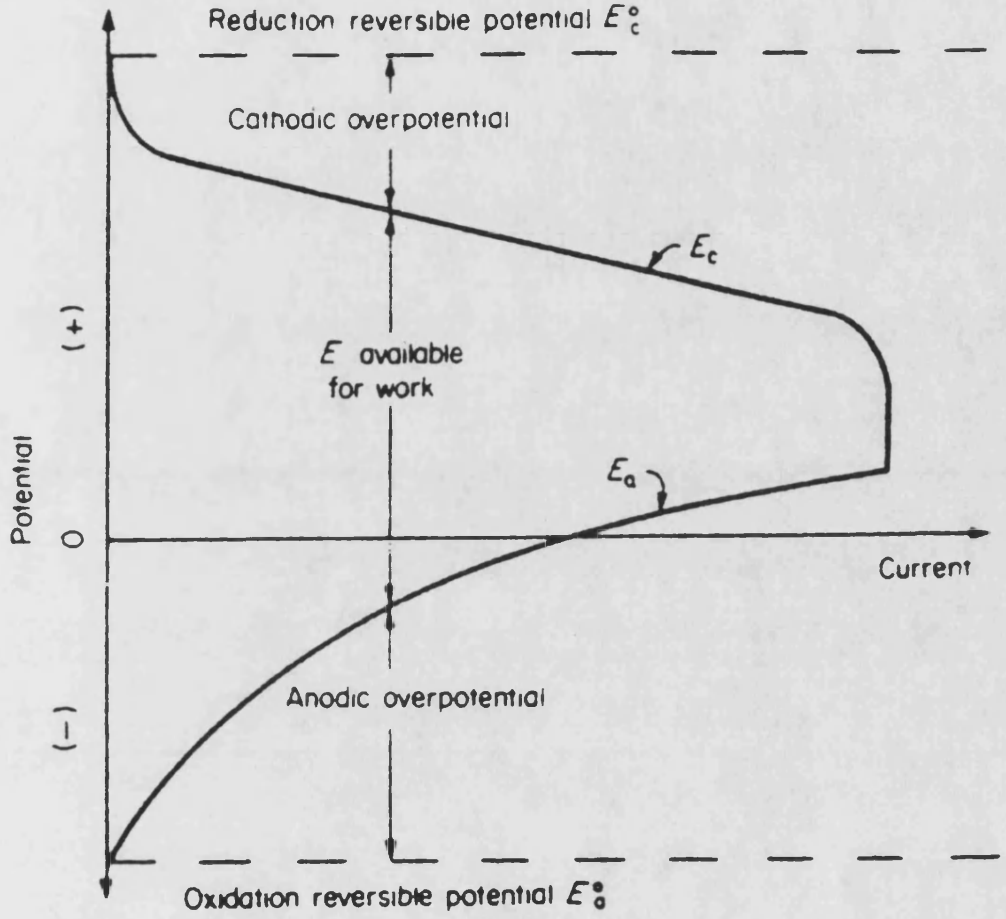


FIGURE 1.4. TYPICAL EXAMPLE SHOWING HOW OVERPOTENTIALS INCREASE WITH CURRENT IN A FUEL CELL. (taken from Wingard et al., 1982)

where O is an oxidised species and R a reduced species. When $a_O = a_R = 1$, $E = E^O$, E^O being defined as the potential of the system when all the species involved in the equilibrium are at unit activity. This potential is called the standard (or normal) potential of the reaction at equilibrium.

Due to the difficulties in obtaining activity values, in practice the equilibrium potential, E, is usually obtained by direct measurement or by extrapolation of a voltage-current plot (figure 1.5) to the potential at zero current - open circuit potential.

1.2.4 Internal Resistance

Voltage-current plots can also be used to obtain a measure of the ohmic overpotential, as the internal resistance of a cell is equivalent to the gradient of the slope of the least squares fit to the experimental curve. Considering figure 1.6:-

$$R\ Total = R_{Ex} + R_{Int}$$

where R Total = total resistance in circuit

R_{Ex} = external load resistance

R_{Int} = internal resistance of cell.

If the voltage recorded when R_{Ex} = infinity is taken as the open circuit voltage, E, then:-

$$I = \frac{E}{R\ Total} = \frac{E}{R_{Ex} + R_{Int}} \quad \text{or,}$$

$$R_{Ex} + R_{Int} = \frac{E}{I}$$

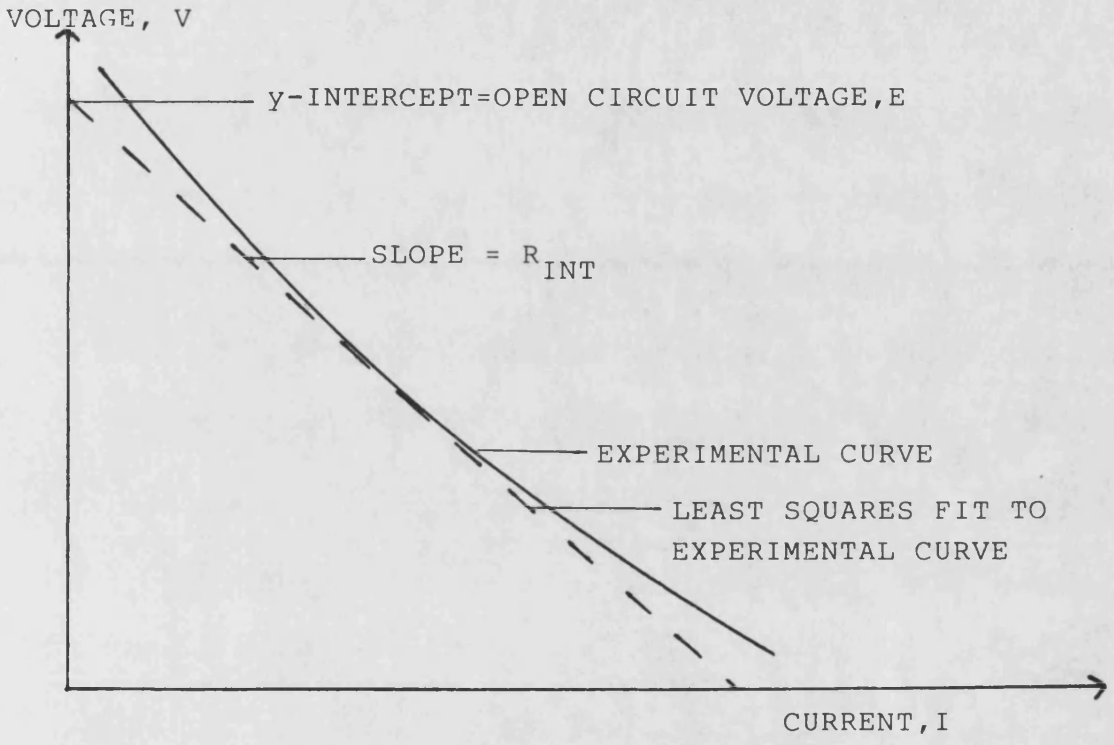


FIGURE 1.5. VOLTAGE-CURRENT PLOT FOR EXPERIMENTAL FUEL CELL

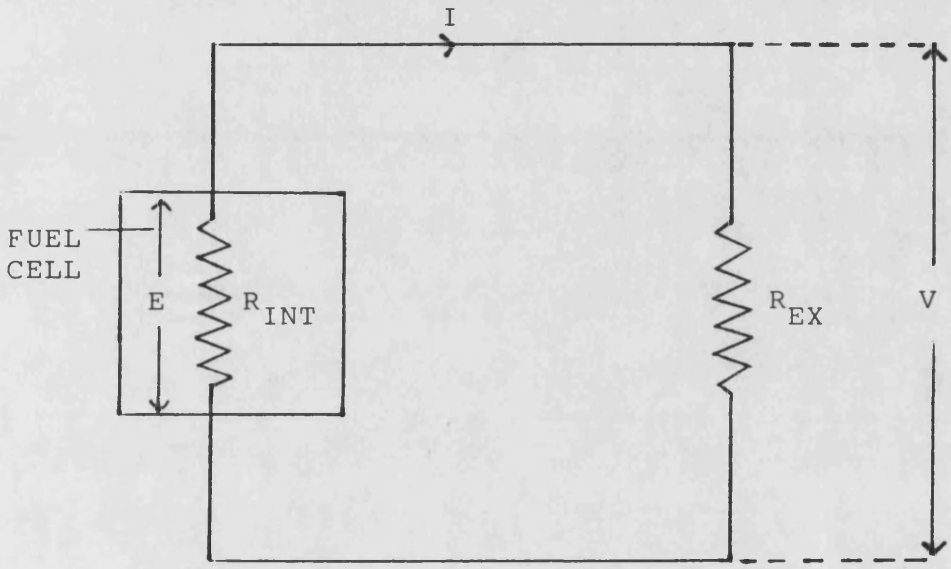


FIGURE 1.6. FUEL CELL CIRCUIT DIAGRAM SHOWING INTERNAL AND EXTERNAL RESISTANCES

substituting $V = IR_{Ex}$ into the above equation and rearranging yields:

$$V = E - R_{Int}I.$$

1.2.5 Current Efficiency

Another indicator of the efficiency of a fuel cell is the current efficiency, ηI, which is defined as:

$$\eta I = \frac{\text{observed current}}{\text{current calculated from rate of consumption of reactant}}$$

In the case of a biofuel cell using an enzyme catalyst the theoretical maximum current, I_{max}, can be calculated from:

$$I_{max} = \frac{A}{60} \times m \times \frac{n}{10^6} \times e \times N_{AV}$$

where A = specific activity of the enzyme (μM products produced/min/mg),

m = mass of enzyme present (mg),

n = number of electrons transferred per mole of product formed,

e = the charge on an electron (1.6×10^{-19} coulombs),

N_{AV} = Avogadro's number (6×10^{23} molecules/mole).

1.2.6 Free Energy Conversion Efficiency

The free energy conversion frequency (ηF) equals the product of current efficiency and voltage efficiency, i.e.

$$\eta F = \eta E . \eta I$$

1.2.7 Coulombic Efficiency

For the measurement of coulombic efficiency the potential of the electrode under study should be effectively constant during operation to ensure that the mechanism of the electrode reaction does not vary. A rigorous method of achieving this is by means of a potentiostat whereby the electrode potential is automatically maintained at a constant value.

The coulombic efficiency, ηc, can be defined as:

$$\eta c = \frac{\text{number of coulombs passed per aliquot of substrate}}{\text{theoretical coulombic yield per aliquot of substrate}}$$

The number of coulombs passed per aliquot of substrate is easily calculated from the product of the current and the duration of the output. The theoretical coulombic yield, C, can be calculated from:

$$C = nFx$$

where n = the number of electrons transferred per mole of product formed,

F = the Faraday constant,

and x = the number of moles of substrate present.

There are several chemical reasons why the current and coulombic efficiencies could be reduced:

(i) Side reactions might occur which yield fewer electron equivalents per mole than the desired reaction.

(ii) Loss or build up of intermediates may occur.

(iii) Non-electrochemical decomposition of the reactants may occur.

(iv) Chemical reaction may occur between the fuel and the oxidant.

1.3 Biofuel Cells

1.3.1 Classification

Biofuel cells can be classified into three types (Higgins and Hill,1985):-

(i) Product type - organisms or enzymes convert electrochemically inactive species to electroactive species suitable for use in a conventional fuel cell, e.g.

$$\text{Glucose} \xrightarrow[\text{bacteria}]{\text{fermentative}} H_2$$

$$H_2 \longrightarrow 2H^+ + 2e^- \text{ (anode)}$$

$$2H^+ + \tfrac{1}{2}O_2 + 2e^- \longrightarrow H_2O \text{ (cathode)}$$

(ii) Depolariser type - organisms or redox enzymes act as catalysts of the electrochemical reactions, e.g.

$$H_2 \xrightarrow{\text{hydrogenase}} 2H^+ + 2e^- \text{ (anode)}$$

$$2H^+ + \tfrac{1}{2}O_2 + 2e^- \xrightarrow{\text{laccase}} H_2O \text{ (cathode)}$$

(in each case the electrode reaction regenerates the enzyme to the appropriate oxidation state).

(iii) Regenerative - organisms or enzymes regenerate redox compounds which in turn carry out the electrochemical reaction, e.g.

$$CH_3OH + H_2O + 2\text{PES (ox)} \xrightarrow[\text{dehydrogenase}]{\text{methanol}} HCOOH + 2\text{PES (red)} + 4H^+$$

$$2\text{PES (red)} \longrightarrow 2\text{PES (ox)} + 4e^- \text{ (anode)}$$

$$4H^+ + 4e^- + O_2 \longrightarrow 2H_2O \text{ (cathode)}$$

In the product type biofuel cell, while the electrochemical conversion may be highly efficient, there are inevitable losses associated with the microbial transformation of the fuel to hydrogen e.g. the high energy input in the form of stirring, gassing and temperature control. The microbial component of the system, however, effectively broadens the range of fuels available to the cell and is particularly suited to the utilisation of dilute aqueous fuels or wastes.

The depolarizer biofuel cell involves a far more intimate connection between the biological and electrochemical systems in that the biological catalyst is used to effect one or both of the electrode reactions. Until recently, this form of bioelectrocatalysis was thought to be of little value, due to the problems in obtaining direct electron transfer between the biological component and the electrode. The development of novel electrode material (Albery et al., 1985) may overcome these problems, but at present such systems have mainly been limited to the biosensor field.

The regenerative type of biofuel cell can be regarded as intermediate between the two classes. Here the biocatalyst is present in the electrode compartment(s) as in the depolarizer cell, but a mediator is used to shuttle electrons between the biocatalyst and the electrode. Mediators are highly electroactive compounds of low molecular weight that undergo reduction at the biocatalyst. They then move to the solid electrode surface by diffusion

and/or convection and readily undergo reoxidation at this surface. This type of cell has proved most popular, but has not quite provided a satisfactory solution to the electron transfer problem (see Section 1.3.3.).

1.3.2 Development

(i) Microbial Fuel Cells

The development of biofuel cells began with the construction of a depolarizer cell by Potter (1910, 1912). Two half cells containing glucose were connected by a salt bridge and the addition of yeast (or E.coli) to one side resulted in an observable current. Potter also noted that enzyme material extracted from micro-organisms could be used to produce similar effects. The first example of a regenerative type cell was described by Cohen (1931) who showed that "poising agents" or mediators (benzoquinone or ferricyanide) could be used to shuttle electrons from glucose metabolising bacteria to an electrode.A "bacterial battery" was constructed with each unit cell yielding 0.2mA at 0.5V for at least 5 minutes, and as was the case in Potter's work, the measurements were made without an effective cathode.

Yudkin (1935) showed that oxidation-reduction ('redox') potentials could be observed at the electrode through traces of electroactive material escaping from intact or lysed organisms, but that more reliable measurements of 'reducing power' were made possible by the inclusion of redox indicators which act as mediators.

These early studies on 'biological potentials' were regarded as mere novelty, and it was not until the 1960's with the boom in space research that there was a renewed interest in bioenergy.

Most of the work on microbial fuel cells in the sixties and seventies was concerned with microbial cells and fermentation broths within the anodic compartment e.g. a bacterial methane fuel cell was reported by van Hees (1965). Pseudomonas methanica was employed to effect electron transfer from methane to the electrode. The device showed free energy efficiencies in the range 8.2-14.2% and a maximum power density of 2.8μWcm^{-2} at 0.35V. Wingard et al,(1982) pointed out that it was unlikely that the majority of such cells operated by direct electron transfer from the catalyst to the electrode. Disalvo and Videla (1981) suggested that changes in dissolved oxygen concentration were responsible for the observed currents, but the more likely cause was the formation of electroactive products such as hydrogen (van Hees, 1965; Karube et al, 1977).

Studies on product cells have continued up to the present time and in some cases have been much more encouraging. Karube et al. (1981) have employed immobilised Clostridium butyricum, generating hydrogen from waste water from an alcohol factory for use in conventional hydrogen-oxygen cells. The fuel cell system (5 cells) gave 0.8A at 2.2V for a 10 day period.

Until recently the development of microbial regenerative biofuel cells has received relatively little attention. However a research group at Queen Elizabeth

College, London have examined a range of anodic systems in the last few years (Bennetto, 1984). Whole micro-organism fuel cells have been developed based on redox mediators which are both rapidly reduced by micro-organisms and rapidly re-oxidised back at the anode. The mechanisms of these microbe-electrochemical interactions are poorly understood, but are likely to involve a "short-circuiting" of electron flow from the electron transport chain.

The most effective fuel cell employed Proteus vulgaris with thionine as the mediator, glucose as the fuel and ferricyanide being reduced at the cathode. Coulombic yields in the region of 30-60%, and current densities of up to 6mAcm^{-2} were obtained (Delaney et al,1984). It was noted, however, that thionine was not sufficiently stable for long-term use. The potential advantage of whole micro-organism mediated fuel cells compared to enzyme based cells lie mainly in the wide range of fuels that can be used by different organisms and the fact that a complete oxidation of the fuel is usually possible. Some of the various studies on microbial fuel cells are shown in Table 1.2.

(ii) Enzyme Fuel Cells

In these devices enzymes have been employed in the anodic and/or cathodic compartment to catalyse the oxidation and reduction reactions. The fuel cells have been mainly of the regenerative type and their development has occured only recently in parallel with the development of techniques to:(i) isolate and purify

Table 1.2 Microbial Fuel Cells (Unless otherwise stated, platinum was used as the anodic electrode material, and oxygen as the cathodic reactant).

Microbial Cells	Anodic Substrate	Mediator	Comments	Reference
Pseudomonas methanica	Methane	-	Cells free in anodic solution	Van Hees, 1965
Micrococcus cerificans	n-Hexadecane	-		Videla and Arvia, 1971
Saccharomyces cerivisiae	Glucose	-	Cells recirculated from fermenter, electrochemical measurements made during log-growth phase	Videla and Arvia, 1975
Micrococcus cerificans	Glucose	-		Disalvo and Videla, 1981
Clostridium butyricum	Glucose	-	Cells immobilised in polyacrylamide gel	Karube et al, 1977

Table 1.2 (continued)

Microbial Cells	Anodic Substrate	Mediator	Comments	References
Clostridium butyricum	Hydrogen	-	Product type cell-cells immobilised in agar acetylcellulose filters using alcohol factory's waste water as fuel.	Karube et al., 1981
Escherichia coli/yeast	Glucose	Thionine/ Resorufin	Cells free in anodic solution. Potassium ferricyanide as cathodic reactant	Benetto et al., 1983
Proteus vulgaris	Glucose	Thionine	As above. Best system obtained from a range of microorganism-mediator-substrate combinations Anodes made of reticulated vitreous carbon	Delaney et al., 1984

stable enzymes and (ii) immobilise such enzymes and cofactors on solid supports. In addition it is hoped that the successful development of depolariser type cells can soon be achieved through advances in procedures for establishing direct electron transfer between enzyme redox centres and electrodes.

The first report of an enzyme fuel cell was in 1962 by Davis and Yarbrough who used methylene blue as a mediator for glucose oxidase in a glucose-powered cell. Mizuguchi et al.(1966) used hydrogen as a fuel with hydrogenase coupled to the anode by either methylene blue or methyl viologen. Laccase was also used to catalyse the cathodic reduction of oxygen using hydroquinone as the mediator. The replacement of laccase with an analogous non-biological system, however, such as ammonium chloride and copper sulphate gave the same effect. Another series of early biochemical fuel cell studies with immobilised enzymes was carried out by Drake (1968), as part of a larger study on the oxidation of glucose for a power source for an artificial heart. Glucose oxidase was immobilised to carbon electrodes using a range of techniques including the co-immobilisation of redox-couplers. Current densities of up to 0.5mAcm^{-2} were achieved,but with poor stability.

Immobilised glucose oxidase was also used as a depolariser fuel cell catalyst by Lahoda et al.(1975). Glucose oxidase was immobilised on platinum screens by three different methods: acrylamide gel, glutaraldehyde, and glutaraldehyde with an electrical charge. Steady

state current densities of 1-15μAcm^{-2} were achieved with the acrylamide gel electode giving the poorest performance - it was suggested that this could have been due to severe internal diffusional resistances to glucose or product transport. The performance variations between electrodes may also have been due to different quantities of immobilised enzyme, to differences in enzyme microenvironment, or to differences in efficiency of electron transfer from the enzyme or cofactor to the external circuit. The poor current densities achieved in this study indicated the difficulties in achieving direct electron transfer from an enzyme to an electrode and it was clear that greater attention to electrode/cell design and the cathode reaction was required.

Poor current densities (1μAcm^{-2}) were also achieved by Weibel and Dodge (1975) using glucose oxidase in a regenerative type cell. Dichlorophenolindophenol (DCPIP) was used as the mediator and the low output was mainly due to the fact that a high resistance salt bridge was chosen to connect the half-cells in order to minimise chemical shorting of the anodic half-reaction sequence by oxygen diffusion from the cathodic chamber. Coulombic efficiencies in the region of 90% were obtained and were thought to be due to the virtual elimination of chemical shorting.

Varfolomeev et al.(1977) employed hydrogenase in a regenerative type cell with methyl viologen as the mediator. The current produced was found to be initially proportional to the amount of enzyme added, but then limited (at a current density of 220μA/cm^2) by the

diffusion of the reduced mediator. Increasing the concentration of the methyl viologen did not give any linear increase in current due to the mediator forming an electrochemically inactive dimer.

More recently a methanol powered cell employing a quinoprotein alcohol dehydrogenase (ADH) from methylotrophic bacteria has been developed (Plotkin et al., 1981; Turner et al., 1982; Davis et al., 1983). The enzyme, purified from Pseudomonas AM1 or Pseudomonas extorquens is of particular interest because as well as methanol it uses other primary alcohols and formaldehyde as substrates (Sperl et al., 1974). Therefore,in the overall reaction in the anodic side of the cell, methanol is converted to formate and four electrons are produced for each molecule of methanol oxidised.

Initial studies with the enzyme purified from Pseudomonas AM1 used phenazine ethosulphate (PES) as the mediator in the anaerobic anodic compartment, separated by a cation exchange membrane, from the cathodic compartment in which oxygen was reduced to water at a platinum gauze electrode. The current produced was found to be directly proportional to the PES concentration up to 1.5mM. Further additions did not increase the current above 3.7mA ($4.8Am^{-2}$ current density, at 10Ω external resistance). The current obtained was also directly proportional to the amount of enzyme added up to about 4mg of protein and further addition did not increase the current above 3.7mA. Mass transport of reduced PES was thought to be the limiting factor on the current output. More rapid stirring or an increase in electrode area were proposed to reduce

this effect. The current efficiency under conditions where enzyme activity was limiting was 90%, i.e., virtually all the methanol-oxidising activity of the enzyme was realised in the current output, and the open circuit voltage of the cell was about 300mV.

The poor stability of the current output, especially at the enzyme concentrations required to give higher currents (the current decayed to half of its maximum value in 1½-2 hours), was shown not to be due to loss of enzyme activity but to the dealkylation of PES to its synthetic precursor, phenazine, a reaction accelerated by the high pH values used in the cell. In addition the PES absorbed onto the cation exchange membrane and could therefore have restricted the flow of hydrogen ions from the anodic compartment to the cathodic compartment.

A number of possible membrane materials were tested for proton, mediator and oxygen permeability. An ideal membrane was not found, but the cation exchange membrane supplied by BDH was found to give the best overall performance.

The oxidation of methanol to formate by ADH theoretically yields just over one half of the possible total free energy change for methanol oxidation. The incorporation of formate dehydrogenase which can also use PES as an electron acceptor was therefore attempted, but without success, due to the difficulty in obtaining conditions, especially of pH, suitable for both of the enzymes. A preliminary attempt was also made to construct

a realistic prototype fuel cell. A 12-compartment cell with reticulated carbon electrodes was assembled requiring aeration of the cathode but no other gassing or stirring. The cell yielded 13.2mA at 0.3V for a 10Ω external resistance but data on the stability of the output were not presented.

The problems encountered when PES was used as the mediator prompted the evaluation of alternative stable soluble electron acceptors including N, N, N', N'-tetramethyl-4-phenylenediamine (TMPD), safranine-O, phenolsafranine, brilliant blue-B, malachite green, brilliant cresyl blue, 1-methoxyphenazine methosulphate and methyl violet-6B. TMPD proved to be the most suitable, remaining stable at the optimum operational fuel cell pH of 10.5 for more than 20 days. The current produced by the fuel cell with enzyme purified form Pseudomonas extorquens was directly proportional to the TMPD concentration up to 2.0mM. Further addition of TMPD however did not increase the current above 0.13mA ($0.2Am^{-2}$ current density at 10Ω external resistance). The current obtained was again also directly proportional to the amount of enzyme added but up to only 0.1mg of protein. Futher additions of ADH did not increase the current above 0.13mA. It was suggested that the rate of mass transport of reduced TMPD was limiting the current as increased rates of stirring resulted in marked increases in current levels. The proposal that, in cells of identical design, mass transport should limit the output of a PES-mediated system at 3.7mA, while limiting that of a TMPD-mediated system at 0.13mA seems unlikely. A more

likely explanation could be that differences in the efficiency of electron tranfer between the enzyme and the mediator or the mediator and the platinum gauze anode were the cause of the differing levels of output.

The current efficiency of the cell under enzyme activity limiting conditions was 73%. Although the current output of the cell was markedly lowered compared to the PES-mediated cell, the output stability was dramatically improved with only a 10% decrease in output over a 24 hour period of continuous operation. The maximum power derived from the cell was 12μW with a power density of $20mWm^{-2}$.

Although the results achieved with the MDH fuel cells were encouraging, they were not regarded as practical devices for energy transfer. The more immediate application for such systems was seen to be as coulometric sensors for the analysis of primary alcohols.

Enzyme fuel cells have also been constructed with the additional purpose of synthesising compounds of commercial interest. Laane et al.(1984b) used glucose oxidase in the anaerobic anodic compartment with glucose as the substrate/fuel and DCPIP as the mediator at pH8.0. In the cathodic compartment, connected to the anodic compartment by a salt bridge, oxygen was reduced to water at a platinum electrode. In order to increase the open-circuit potential, and hence the driving force for the combined reactions, the pH of the cathodic vessel was brought to pH2.7. In normal operation currents of approximately 350μA ($0.47Am^{-2}$ current density) were

produced but under optimum conditions current >1mA could be reached. The open circuit voltage of the cell was 0.6V.

Glucose oxidase catalyses the oxidation of glucose to glucono-1,5-lactone which then hydrolyses to gluconic acid, a valuable industrial chemical currently produced by fermentation (Komineik, 1983). Measurements showed that glucose was converted exclusively and completely into gluconic acid. Routinely the current was stable for 3½ hours, then depletion of substrate caused a rapid drop in current. No attempt was made to assess the long term current stability.

Xanthine oxidase was used in place of glucose oxidase in the same system (but with xanthine as the fuel) and produced similar results. However, uric acid, the product of xanthine oxidase action, was further oxidised electrochemically in an n = 2 reaction to a diimine intermediate, which then reacted further to give unknown products.

The cathodic reaction was also shown to produce (bio)chemicals: using a gold instead of a platinum electrode, H_2O_2 was generated and subsequently scavenged by chloroperoxidase, which in the presence of Cl^-, will halogenate many compounds. Using barbituric acid as a model substrate, 5-monochloro- and 5,5, dichlorobarbituric acid was expected to be produced, but only the monohalogenated compound was formed. It was observed that the second chlorine atom was removed by a reductive electrochemical reaction. The maximum current produced by this system was 60μA, decaying by 50% over three

days. The poor output of the cell was compensated for by the fact that product formation occured in both compartments - 10mg gluconic acid and 8mg 5-chlorobarbituric acid. A suumary of some of the recent work on enzyme fuel cells is shown in table 1.3.

1.3.3 Electron Transfer in Enzyme Fuel Cells.

At present, the only route to higher current densities is via low molecular weight diffusible mediators linking enzyme redox centres to the electrode. Apart from the fact that mediators are often unstable there are two major problems associated with their use. Firstly most of the mediators employed will react with oxygen. Therefore if the anodic compartment is not kept strictly anaerobic substantial power losses occur because electrons are passed to oxygen as well as to the electrode. These losses are especially difficult to avoid, as the most common cathodic reaction is the reduction of oxygen to water, and the proton permeable membranes that are commonly used to separate the anodic and cathodic compartments also allow diffusion of oxygen (Turner et al, 1982). Secondly the use of redox compounds as intermediates in the electron transfer from substrate to electrode reduces the net reversible potential difference, since the redox potential seen by the electrode is that of the last component of the chain (Aston and Turner, 1984).

An alternative to conventional oxygen acceptors is the use of ferrocene and its derivatives. Ferrocenes, previously largely ignored, presumably because of their

Table 1.3 Summary of Recent Studies on Enzyme Fuel Cells (Unless stated, platinum was used as the anodic electrode material, and oxygen as the cathodic reactant).

Enzyme	Mediator	Performance	Comments	Reference
Glucose Oxidase	-	Current density 1-15μAm^{-2}	GOD immobilised on platinum screen using acrylamide gel or glutaraldehyde	Lahoda et al, 1975
Glucose Oxidase	DCPIP	Current density 1μAcm^{-2} Coulombic Efficiency 90%	High resistance salt bridge used to connect half cells	Wleibel and Dodge, 1975
Hydrogenase	Methyl Viologen	Current density 220μAcm^{-2}	Graphite anode. Current limited by diffusion of reduced mediator	Varfolomeev et al, 1977
Alcohol Dehydrogenase	PES	Current density 480μAcm^{-2} current efficiency 90%	Mediator unstable	Plotkin et al, 1980 Turner et al, 1982

Table 1.3 (continued)

Enzyme	Mediator	Performance	Comments	Reference
Alcohol Dehydrogenase	TMPD	Current density $20\mu Acm^{-2}$ Current Efficiency 73%	Stable system, but low output	Davis et al., 1983
Glucose Oxidase	DCPIP	Current density $47\mu Acm^{-2}$	Total conversion of glucose to gluconic acid	Laone et al., 1984b
Xanthine Oxidase	DCPIP	Current density $47\mu Acm^{-2}$	Further electrochemical oxidation of uric acid	Laone et al., 1984b
Glucose Dehydrogenase	Meldola Blue	Current density $200\mu Acm^{-2}$	Product type cell, with mediator immobilised on graphite anode, and with a simulated cathode	Persson et al., 1985

apparent insolubility, are electroactive organometallic compounds acting as pH-independent reversible one electron donors (Higgins et al., 1982). Various derivatives are available (i.e. with various substituents on the ring structure, possibly in polymer form) differing in redox potential and aqueous solubility. The advantage of ferrocenes is that they remain stable in the reduced form, i.e., do not undergo autooxidation, and thus have been used in various configurations as mediators in enzyme electrodes for biosensors (Higgins et al., 1982; Cass et al., 1984) and biofuel cells (Higgins et al., 1984). The restraint imposed by loss of potential, as discussed earlier, still exists and further work is required to find ferrocene derivatives offering fast electron transfer coupled with low redox potential and suitable physical characteristics.

It has been suggested that the immobilisation of cofactors, which serve as the actual electron and proton transfer agents for oxidoreductase enzymes, could be one approach to overcome the problems of electron transfer in enzyme fuel cells (Wingard, 1978; Wingard et al., 1982). Riboflavin has been linked to carbon electrodes retaining its electroactivity (Wingard, 1980) and flavin adenine dinucleotide (FAD) has been linked to graphite electrodes (Sonawat et al., 1984; Phadke et al., 1984). Immobilisation of the glucose oxidase apoenzyme on the FAD modified electrode led to direct electron transfer between the enzyme active site and the electrode, but as yet there has been no report of such an electrode system being employed in a fuel cell.

Therefore, the suitability of this approach for achieving high current densities has yet to be confirmed. It is likely that the size of the enzyme molecules would limit the packing density, but it has been suggested that this limitation may be overcome by the development of sophisticated conducting polymers having conducting "molecular stalks" of differing lengths to which enzyme molecules could be attached (Higgins and Hill, 1985).

Chemical modification of an electrode has produced promising results in the development of a biofuel cell by Persson et al. (1985). Glucose dehydrogenase was immobilised onto porous glass catalysing the conversion of glucose to gluconic acid with the concomitant reduction of NAD to NADH. The reactant solution was then passed into an anodic half-cell where the NADH was oxidised at a graphite electrode, modified by the absorption of N, N-dimethyl-7-amino-1, 2-benzophenoxazinium (Meldola Blue). A current density of $0.2mAcm^{-2}$ at a cell voltage of approximately 0.8V was obtained for more than 8 hours with a simulated oxygen cathode (A potentiostatic circuit was used to provide a potential identical to that of an ideal oxygen electrode). The internal resistance of the cell was high - around 200Ω - indicating that an improvement in cell design was required. For practical application a more conventional cathodic reaction would have to be employed and it is likely that this would reduce the cell's output. In addition, for prolonged use the stability of the immobilised mediator would have to be improved.

It is clear that there will be many new developments

in electrode modification, and probably, electrode materials e.g. the use of conducting salts as electrode materials for a glucose oxidase biosensor (Albery et al., 1985). It has still to be determined whether or not such developments will give electron transfer suitable for the attainment of a practical range of current densities in a biofuel cell.

1.3.4 Applications of Biofuel Cells

At present biochemical fuel cells are far from being used as commercial power generators; their more immediate application has been in the analytical mode as biosensors in response to the need for sensitive monitoring of a wide range of substances. There has been a rapid acceleration in biosensor research in the 1980's as reviewed by Lowe (1984), Gronow et al. (1985), and Van Brunt (1987). The view is that biosensors will soon be widely used in clinical analysis, health care, veterinary and agricultural applications, industrial processing and monitoring , and environmental and pollution control.

The development of biofuel cells, as such, is likely to be directed towards meeting specific requirements e.g. (i) small scale electricity generation in isolated situations, especially in some Third World countries where various plant and animal wastes could be used as fuels (Aston and Turner, 1984).

(ii) the removal of human waste materials from a closed ecology such as on extended space flights where carbon dioxide, urine and faecal material could be transformed

ideally, into electricity, oxygen and food (Glazebrook and Jones, 1966).

(iii) a micro glucose electrode conveniently implanted in the body could serve as an excellent power source for a pacemaker, generating low power pulses from plasma glucose (Drake, 1968).

(iv) deriving power without producing heat or noise, such a power source, which could be difficult to detect, may have military applications e.g. a battery recharger operating at ambient temperatures using readily available diesel, kerosene, or methanol/water anti-freeze mixtures as fuels (Higgins and Hill, 1985).

(v) the production of electricity during the detoxification of industrial waste waters (Suzuki et al, 1980a,b).

(vi) the synthesis of various (bio)chemicals of commercial interest (Laane et al., 1984a,b).

1.3.5 Enzyme Immobilisation

Immobilised enzymes have been employed in many biofuel cells (and especially biosensors) with great success. The technology of immobilising biological materials to various natural and man-made materials has advanced considerably in the last 20 years. The techniques for enzyme immobilisation have been extensively reviewed e.g. Zaborsky (1973), Mosbach (1976), Chibata (1978) and Guilbault and Sadar (1978) and can be summarised as follows:-

(i)Adsorption. Adsorption is the oldest immobilisation technique. The enzyme is attached to a surface-active

material such as charcoal, alumina, clay, glass or ion-exchange resins. Attachment of the enzyme to a support is mainly due to Van der Waals interactions, hydrogen bonding and salt linkages. As these are weaker, more easily dissociable complexes than covalent bonds, distortion and inactivation of the enzyme are probably less likely to occur. The major disadvantage of this method, is that desorption of enzymes can occur with variations in pH, ionic strength, temperature, solvent, substrate or product concentration.

(ii) Covalent Attachment This is a commonly used technique. Nonessential amino acid residues of the enzymes are attached to chemically activated supports such as cellulose, glass or synthetic polymers. Enzyme-support linkage has been made via azide, isocyanate, carbodiimide and other derivatives of the supports. Covalent bonds provide the most stable linkage.

(iii) Cross-linking Intermolecular covalent linkages can be formed between molecules of the enzyme with the aid of bifunctional agents such as diisothiocyanate, bisimidates, alkylating agents and dialdehydes. The major disadvantage of this technique is that many enzymes are sensitive to the coupling reagents such that they loose activity in the process of crosslinking.

(iv) Entrapment Materials like polyacrylamides, silica gel, and starch are cross-linked in the presence of enzyme. Thus the enzyme is captured within the polymer matrix while substrates and products diffuse freely. The disadvantages of this method are the leakage of

enzymes from the polymeric network and the inactivation of enzymes by the free radicals generated during the initiation of polymerisation. In addition, in practice there is always some limitation on the diffusion of substrates to, and products from, the enzyme.

(v) Microencapsulation Enzymes are trapped by membranes of various polymers, usually in capsules with mean diameters ranging from 5 to 300μm. The enzyme is too large to move through the pores of the semi-permeable synthetic membrane, but smaller substrate and product molecules can move back and forth through the pores of the membrane readily. The major disadvantage of this method is that successful encapsulation of the enzyme requires very high protein concentrations. Also, as with entrapment, there is always some limitation on the diffusion of substrates to, and products from, the enzyme.

The major advantage of using immobilised rather than soluble enzymes in practical applications is that the expensive catalysts can be recovered, resulting in substantial cost reduction. In addition the enzymes are usually more stable because they are placed in a more natural environment - in vivo the majority of enzymes are attached to cell surfaces or entrapped within cell membranes (Guilbault and Sadar, 1978).

The disadvantages of immobilised enzymes, especially concerning their use in biofuel cells are loss of activity on immobilisation, limiting packing densities due to the actual size of the enzyme molecules or the structure of the support materials, and limitations on the transport

of reacting molecules.

For a regenerative type fuel cell employing an immobilised enzyme the sequence of steps that make up the overall electron transfer reaction are probably as follows (adapted from Wingard, 1978):-

(i) transport and complex formation between non-immobilised and any immobilised species (substrate, mediator, cofactor and enzyme).

(ii) reaction at active site

(iii) transport of mediator to electrode surface

(iv) electron transfer at electrode surface.

The rate of step (iii) can usually be optimised by immobilising the enzyme to the electrode itself, and as discussed in section 1.3.3 direct electron transfer could be achieved by immobilising the enzyme to the electrode via its cofactor.

1.3.6 Background to the Project

Preliminary experiments (Yue and Lowther, 1986) had indicated that a carbon fabric could be used as an electrode material in a biofuel cell. Promising current densities (up to $0.55Am^{-2}$) were achieved using the enzymes alcohol oxidase and formate dehydrogenase with methanol or formaldehyde as the substrate and phenazine methosulphate (PMS) as the mediator. Unfortunately the stability of the current output was poor and was thought to be due to localised denaturing of enzyme or mediator instability. Enzyme immobilisation, by adsorption to the carbon cloth was shown to increase the performance

of the fuel cell.

The objectives of the project were:-

(i) to improve the design of the system so as to obtain high current densities and to enhance the stability of the power output.

(ii) to elucidate the mechanism of the bioelectrocatalytic processes involved in the operation of biofuel cells, and the factors which influence the kinetic and transport rates.

In relation to the first objective the aspects of fuel cell operation studied were:- enzyme/mediator suitability, enzyme immobilisation, cell and electrode design, pH control and the cathodic reaction.

The low cost, high surface area, excellent adsorption and electrical properties (- ideal for efficient electron transfer) and physical stability of the carbon cloth (see Appendix II) makes it a possible alternative to the more conventional electrode materials. The project aimed therefore to assess the potential of the cloth for use in regenerative biofuel cells.

For the initial experiments a biofuel cell system similar to that employed in the preliminary studies was used. The basic reaction scheme is shown in Figure 1.7. In the anodic compartment alcohol oxidase oxidises methanol and reduces the redox mediator which is reoxidised at the anode. Electrons flow from the anode through the external circuit to the cathode, where oxygen is reduced. The electrical circuit is completed by protons, derived from the enzymic reaction, diffusing across

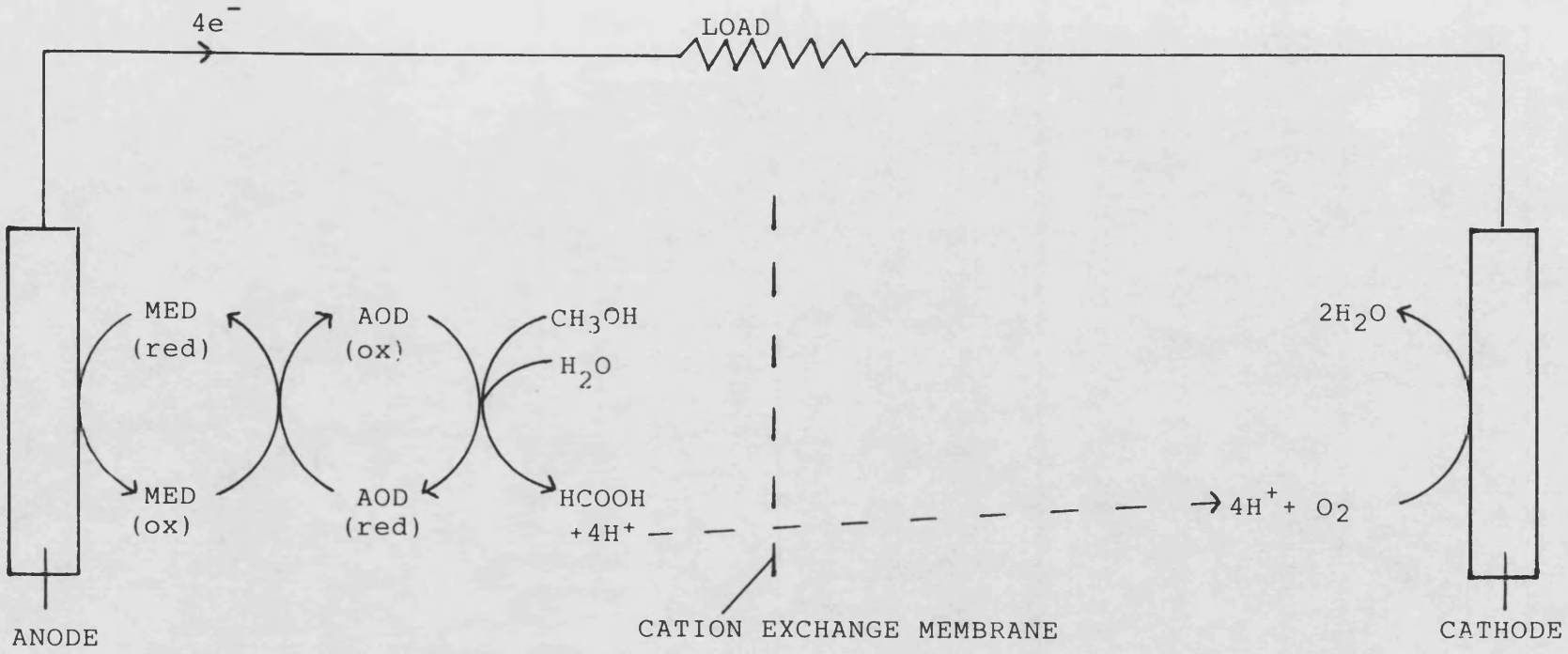


FIGURE 1.7. SCHEMATIC REPRESENTATION OF A BIOFUEL CELL RUNNING ON METHANOL

the membrane separating the two halves of the cell to react at the cathode. PES was prefered to PMS as the mediator due to its greater stability (Ghosh and Quayle, 1978).

This type of cell is particularly interesting since (as with ADH) methanol is oxidised to formaldehyde and then on to formate, by the single enzyme, with the release of a total of four electrons. (It should be noted, however, that the enzyme activity with formaldehyde is slightly lower than that with methanol, Yue and Lowther, 1986). The advantage of a potentially large release of energy using this system is complemented by the suitability of methanol as a fuel, being cheap, stable, easily transportable and readily available (McDougall, 1976).

2 MATERIALS and METHODS

2.1 Materials

MilliQ purified water was used for all buffer and electrolyte solutions. All laboratory reagents were of Analar grade or the highest purity readily available and were purchased from B.D.H. Ltd., Poole, Dorset. Specialised equipment and chemicals were purchased from the following manufacturers :-

Aldrich Chemical Co. Ltd. (Gillingham, Dorset)

Acetylferrocene

2,2' Azinobis (3-ethyl-benzothiazoline-6-sulphonic acid) Diammonium salt. (ABTS)

N,N,N',N'-tetramethyl-4-phenylenediamine (TMPD)

B.D.H. Ltd.(Poole, Dorset)

Cation Exchange membranes (125mm x 125mm sheets) (Appendix I)

2,6-Dichlorophenolindophenol (DCPIP)

Methylene blue (MB)

Polyethyleneimine

Hydrolysed starch

Toluidine blue-0

Brownash Engineers Ltd. (Ashtead, Surrey)

Carbon cloth, grade C.S.A. (Appendix II)

Boehringer Corporation Ltd. (Lewis, Sussex)

Horseradish peroxidase

Yeast alcohol dehydrogenase (YADH)

Chloride Industrial Batteries Ltd. (Bristol)

Graphite rods (7mm diameter)

Electroplan. (Royston, Herts.)

Fluke 8022B digital monitors

Fluorochem.Ltd.(Glossop, Derbyshire)

1,4-Benzoquinone (BQ)

GEC Elliot Process Instruments Ltd. (Croydon, Essex)

Gas flow ratemeters (0-40cm^3/min range)

Internation Products Corporation (Chiselhurst, Kent)

Micro detergent

Jay-Jay Instruments Ltd.

Resistance Box (0.1Ω to 10,000Ω)

Johnstone and Mathey Chemicals (Royston, Herts)

Platinum gauze

Loctite (U.K.) Ltd. (Welwyn Garden City, Herts)

Clear silicone sealant

Pall Process Filtration Ltd. (Portsmouth)

Biodyne A membrane

Radiometer (Copenhagen, Denmark)

Calomel electrode, Type K401

R.S. Components Ltd. (Corby, Northants)

Silver loaded epoxy adhesive

Sigma Chemical Co. Ltd. (Poole, Dorset)

Alcohol oxidase (AOD) (from Candida boidinii)

Basic Blue 24

Bovine serum albumin (BSA)

Carrageenan, Kappa

o-Dianisidine

Ferrocene monocarboxylic acid (FMCA)

Glucose oxidase (GOD) (from Aspergillus niger, Type X)

Glutaraldehyde (electron microscopy grade)

Methyl viologen (MV)

Nicotinamide adenine dinucleotide (NAD)

Phenozine ethosulphate (PES)

Thionine (Acetate salt)

YADH attached to beaded agarose

Thompson Electrochem Ltd. (Newcastle)

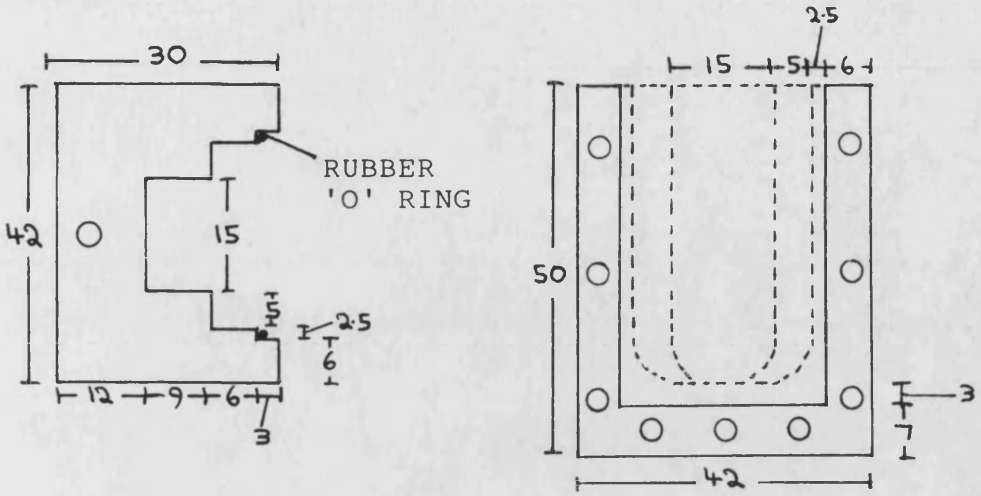
Ministat potentiostat, Model No. 251

2.2 Cell Design

The experimental fuel cell was made of perspex to the specifications shown in figure 2.1. When fully assembled the cell comprised an anodic compartment and a cathodic compartment - of 10ml volume - separated by a cation exchange membrane. The membrane was sealed inside its frame with silicone rubber, and was then clamped between the two halves of the cell body by metal bolts. The contents of the compartments were mixed using small magnetic stirrers.

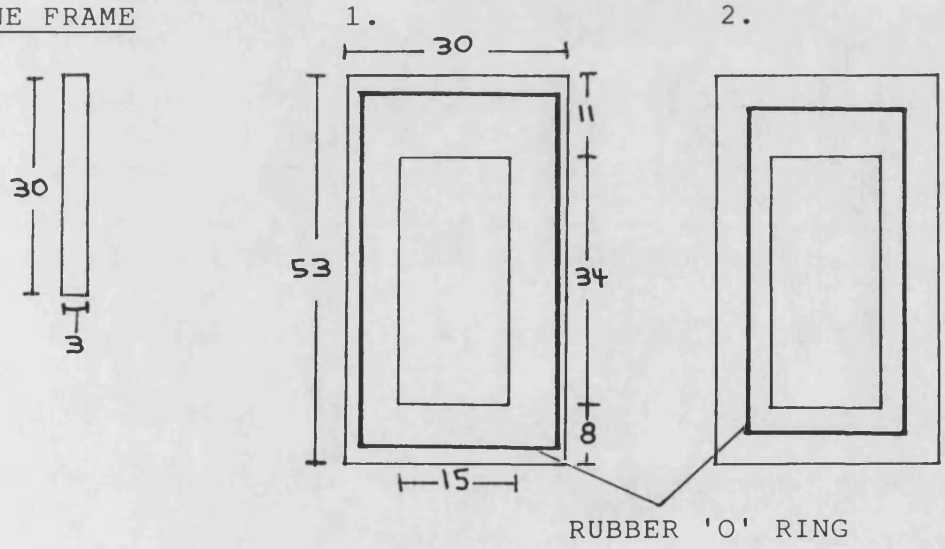
Both the anode and cathode consisted of a carbon cloth, 4.0 x 3.0 cm, attached to a graphite rod, 6cm length, 0.7cm diameter. The cloth was attached to the rod either by (i) a small plastic clip, or (ii) silver loaded epoxy adhesive. The two methods of attachment produced similar electrical conductivity, but the former was preferred allowing easier replacement of the cloth, which often became frayed after repeated use. Before use, the electrodes were boiled in 1M sodium hydroxide for 2 hours, then rinsed several times with 1M hydrochloric acid and MilliQ purified water, to clean the electrodes and produce a reproducible electrode surface. In the early experiments the electrodes were connected to the external circuit via crocodile clips. However, it was found that brass rings held onto the graphite rods by a grub screw provided a better connection. The external circuit is shown in figure 2.2. Digital multimeters were used to measure the voltage and current produced

CELL BODY



TOP VIEW FRONT VIEW

MEMBRANE FRAME



COMPARTMENT COVERS

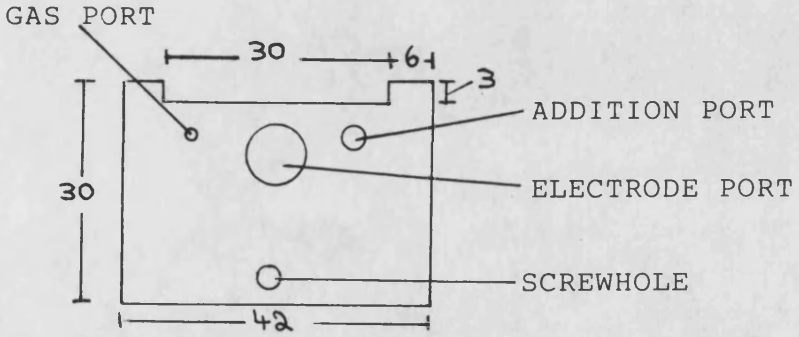


FIGURE 2.1. CELL DESIGN (NUMBERS REPRESENT MEASUREMENTS IN MM)

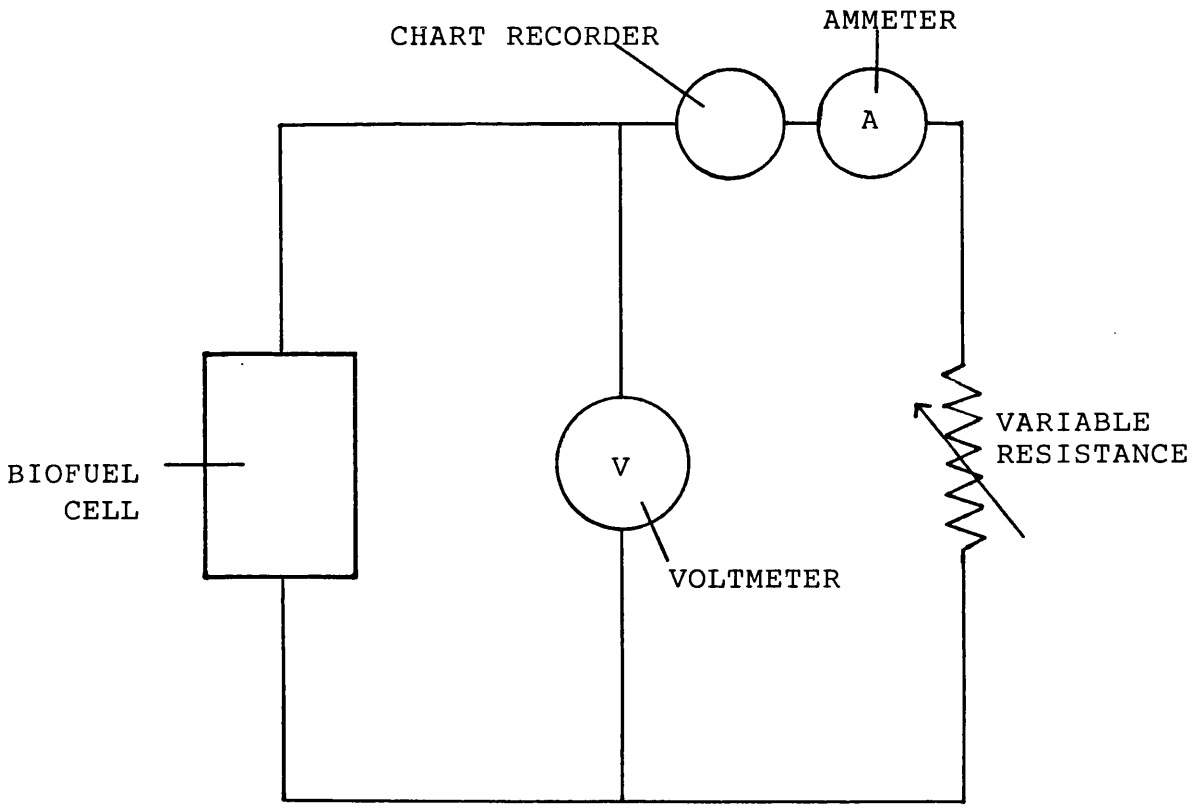


FIGURE 2.2. EXTERNAL CIRCUIT FOR BIOFUEL CELL

by the fuel cell. The time courses of the fuel cell outputs were measured against an external resistance of 10 ohms and monitored using a chart recorder.

Platinum electrodes were also constructed for use in the experimental fuel cell. A 5cm² platinum gauze (52 mesh, woven from 0.1mm diameter wire) was cut into two halves. Each was then rolled into a cylinder (8mm diameter) with strengthening sections of platinum wire at the top and bottom, and a 2cm length of wire spot welded on the top for connection to the external circuit. Before use in the fuel cell the electrodes were soaked in concentrated nitric acid overnight, then rinsed thoroughly with MilliQ purified water.

A modified glass cell was constructed suitable for use under the control of a potentiostat; figure 2.3. The potential of the working electrode was controlled using a Ministat potentiostat and measured with a multimeter. The current flowing between the working and the counter electrode was measured using a multimeter and chart recorder.

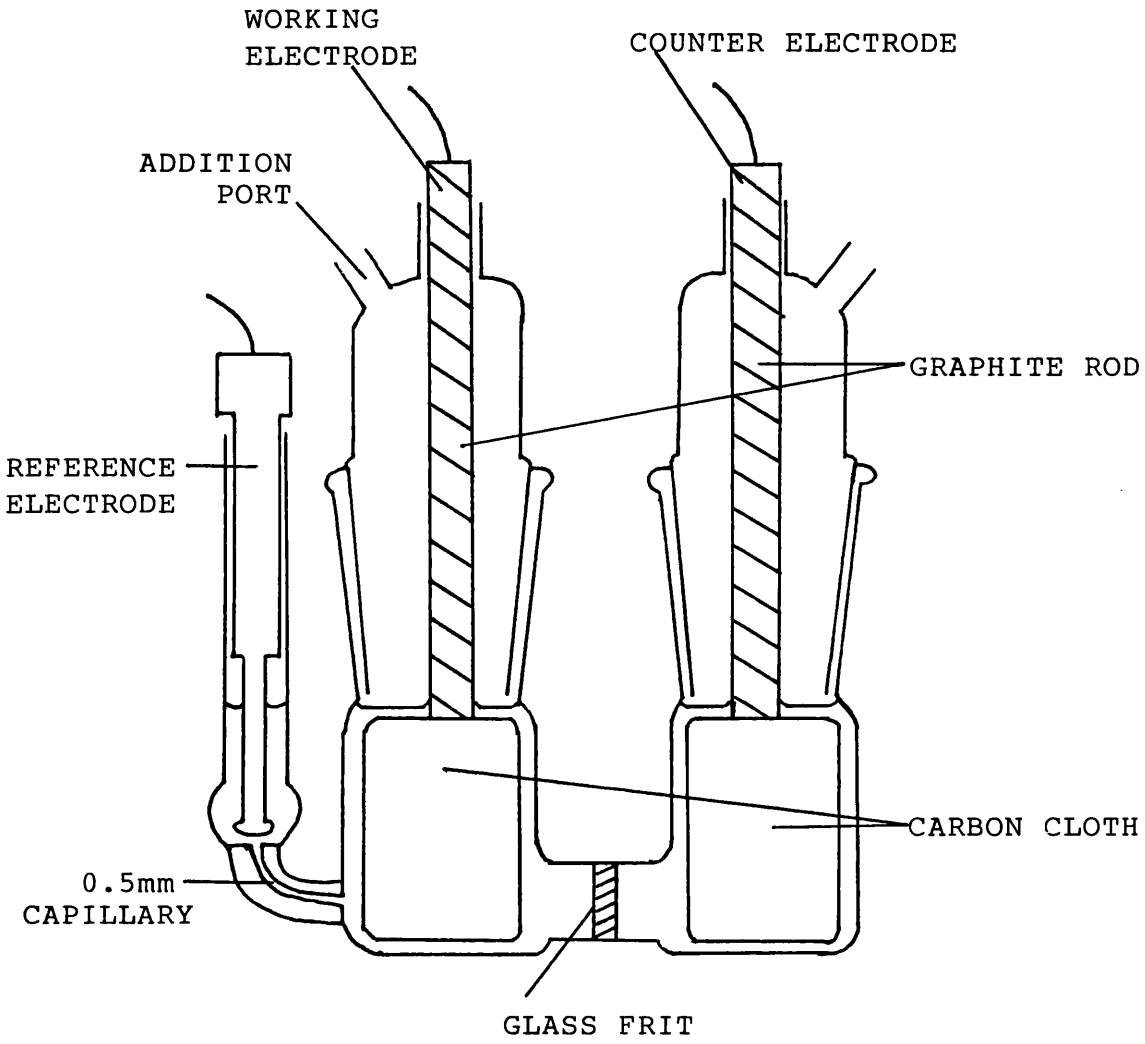


FIGURE 2.3. CELL FOR EXPERIMENTS UNDER POTENTIOMETRIC CONTROL

(drawn to scale)

2.3 Preparation of Hydroxyethylferrocene

The method used was adapted from that of Little and Eisenthal (1961) for the preparation of 1-(α-Hydroxybenzyl)-1'-carbamylferrocene from 1-benzoyl-1'-diphenylcarbamylferrocene. A warm solution of 2.5g acetylferrocene in 100ml methanol was treated with 0.1ml of 10% sodium hydroxide in a 500ml flask. The solution was stirred and 25ml of a 10% potassium borohydride solution, in 10% aqueous sodium hydroxide, was added in one portion. The reaction mixture was stirred for 30 minutes at room temperature and 4 hours 30 minutes at gentle reflux. At the end of the reaction a fine rust coloured powder was observed in the vessel, 15ml of acetone was added and the solution left at at 4°C overnight. Most of the solution was then removed on a rotovac leaving an orange sludge. Water (20ml) and 100ml of dichloromethane (DCM) were added followed by extraction in a separating funnel. The aqueous phase still retained some colour; therefore this was extracted with a further 50ml of DCM. The pooled organic phase was then washed twice with water and dried over anhydrous sodium sulphate. The DCM was then removed from the filtrate on a rotovac, leaving orange/rust crystals. The product was then recrystallised from petroleum ether (60-80°C).

Melting point acetylferrocene	= 78.5-79°C	Reported value 81-83°C
Melting point product	= 72.5-74°C	Reported value 73-75°C
Mixed melting point	= 47.0-55°C	

Thin layer chromatography was then performed on the acetylferrocene and the product giving R.F. values of 0.48 and 0.44 respectively. Confirmation that the product was hydroxyethylferrocene was obtained from C^{13} nuclear magnetic resonance scans. 1.69g of HEF were produced giving a yield of 67.6%.

2.4 Enzyme assays

The enzyme activities were measured in units. One unit of enzyme catalyses the transformation of 1µmol substrate per minute under assay conditions.

Alcohol oxidase (EC 1.1.3.13 Alcohol:oxygen oxidoreductase) from Candida boidinii (lyophilised powder) was assayed using a modification of the method by Sahm and Wagner (1973) given by Bergmeyer (1983).

Reaction scheme:-

$$\text{Methanol} + O_2 \xrightarrow{\text{AOD}} \text{formaldehyde} + H_2O_2$$

$$H_2O_2 + DH_2 \xrightarrow{\text{POD}} 2H_2O + D$$

AOD = alcohol oxidase; POD = peroxidase; DH_2 = leuco dyestuff; D = dyestuff.

The assay mixture contained:-

2.80ml phosphate buffer/ABTS (0.25mol/l; pH7.5; ABTS 2mmol/l)

0.05ml enzyme in buffer

0.01ml POD (10mg/ml)

0.01ml H_2O_2 (0.1ml 30% H_2O_2/l)

0.10ml methanol (0.01ml/ml)

The increase in the absorbance at 405nm was measured using a Pye Unicam SP6-450 spectrophotometer. The extinction coeficient for ABTS, ϵ = 3.681 $mmol^{-1}cm^{-1}$; final volume 2.97ml; 20°C.

Yeast Alcohol Dehydrogenase (EC 1.1.1.1. Alcohol:NAD oxidoreductase, lyophilised powder) was assayed using a modification of the method of Backlin (1958).

Reaction scheme:-

$$C_2H_5OH + NAD^+ \underset{\leftarrow}{\overset{YADH}{\rightleftharpoons}} CH_3CHO + NADH + H^+$$

The assay mixture contained:-

0.5ml pyrophosphate buffer, 0.06M. pH8.5

0.1ml NAD, 0.015M

2.2ml distilled water

0.1ml ethanol, 3M

0.1ml enzyme in buffer

The increase in absorbance at 340nm was measured using a Pye Unicam SP6-450 spectrophotometer. The extinction coeficient for NADH, $\varepsilon = 6.22 \times 10^3\ 1ml^{-1}cm^{-1}$. Final volume 3.0ml; 20°C.

Glucose oxidase (EC 1.1.3.4, β-D- Glucose:oxygen 1-oxido reductase) from Aspergillus niger (lyophilised powder) was assayed using a modification of the method of Swoboda and Massey (1965)

Reaction scheme:

$$\beta\text{-D-Glucose} + 0_2 + H_20 \xrightarrow{GOD} \text{D-Gluconic acid} + H_20_2$$

$$H_20_2 + \underset{\text{(reduced, colourless)}}{\text{o-Dianisidine}} \xrightarrow{POD} \underset{\text{(oxidised, red)}}{\text{o-Dianisidine}} + H_20$$

GOD = glucose oxidase

The assay mixture contained:-

2.40ml phosphate buffer/dianisidine (0.25mol/l; pH8.0; dianisidine 0.2mmol/l)

0.50ml glucose (10% w/v)

0.10ml peroxidase (60 Purpurogallin units/ml)

0.10ml enzyme in buffer

The increase in absorbance at 500nm was measured using a Pye Unicam SP6-450 spectrophotometer. The extinction coeficient for oxidised o-dianisidine, $\varepsilon = 7.5 \times 10^3 \text{l mmol}^{-1} \text{cm}^{-1}$. Final volume 3.1ml; 20°C.

3 RESULTS

3.1 Configurations with Alcohol as Fuel

3.1.1 Alcohol oxidase - In free solution and Absorbed on Cloth

For the configuration with AOD in free solution the anodic compartment contained 9ml of electrolyte - 0.2M H_3BO_3, 0.05M NH_4Cl buffer, pH8.0; 5mM methanol, 2.5mM PES and 5mg AOD (0.3 units, Km = 0.4mM). The cathodic compartment in this and all further fuel cell configurations in this section contained 9ml electrolyte,and was gassed with oxygen. Nitrogen (oxygen free) was passed through the anodic compartment in all the configurations in this section.

A maximum current of 475μA was produced which decayed to half this value in 10 minutes (i.e. $t_{\frac{1}{2}}$ = 10 minutes). The addition of further aliquots of substrate and PES had no effect on the rapid drop in current, but the addition of a further 5mg of AOD did produce a temporary resurgence. This suggested that the loss of output was due to denaturation/deactivation of the enzyme - an effect which could possibly be remedied by immobilisation of the enzyme.

The most effective enzyme immobilisation was obtained by pipetting 1ml 0.2M H_3BO_3, 0.05M NH_4Cl, buffer pH8.0, containing 10mg of enzyme onto the cloth, then covering the cloth with a further 14ml of buffer and incubating overnight at 4°C. Under these conditions approximately 0.06 units of enzyme (calculated by measuring the rate of oxygen consumption of $1cm^2$

sections of cloth in a Rank oxygen electrode) were absorbed onto the cloth. After incubation the cloth was rinsed in buffer and placed in the anodic compartment containing electrolyte, methanol and PES as above.

A current of 750μA was produced with a $t_{\frac{1}{2}}$ of 27 minutes. There was therefore some increase in the stability of the cell's output but this was still a very poor performance compared to the results obtained by other workers using similar configurations. To see if this effect was peculiar to alcohol oxidase a slightly different configuration was chosen using yeast alcohol dehydrogenase (fig. 3.1).

3.1.2 YADH in Free Solution and Absorbed on Cloth

For the configuration with YADH in free solution the anodic compartment contained 9ml electrolyte - 0.06M pyrophosphate buffer, pH8.5; 100mM ethanol, 0.5mM NAD, 2.5mM PES and 6 units of YADH (Km = 17mM). The maximum current produced was 570μA with a $t_{\frac{1}{2}}$ of 40 minutes. Further additions of ethanol, PES and NAD had no effect on the current drop, but sequential additions of enzyme maintained a fairly level current for several hours. The experiment was then repeated, monitoring enzyme activity. Figure 3.2 shows that the drop in current output was seemingly due to the parallel loss of enzyme activity.

Enzyme absorption was carried out as in 3.1.1 using 40 units of YADH, and the electrode placed in the anodic compartment containing ethanol, NAD and PES as above. The maximum current produced was 550μA with

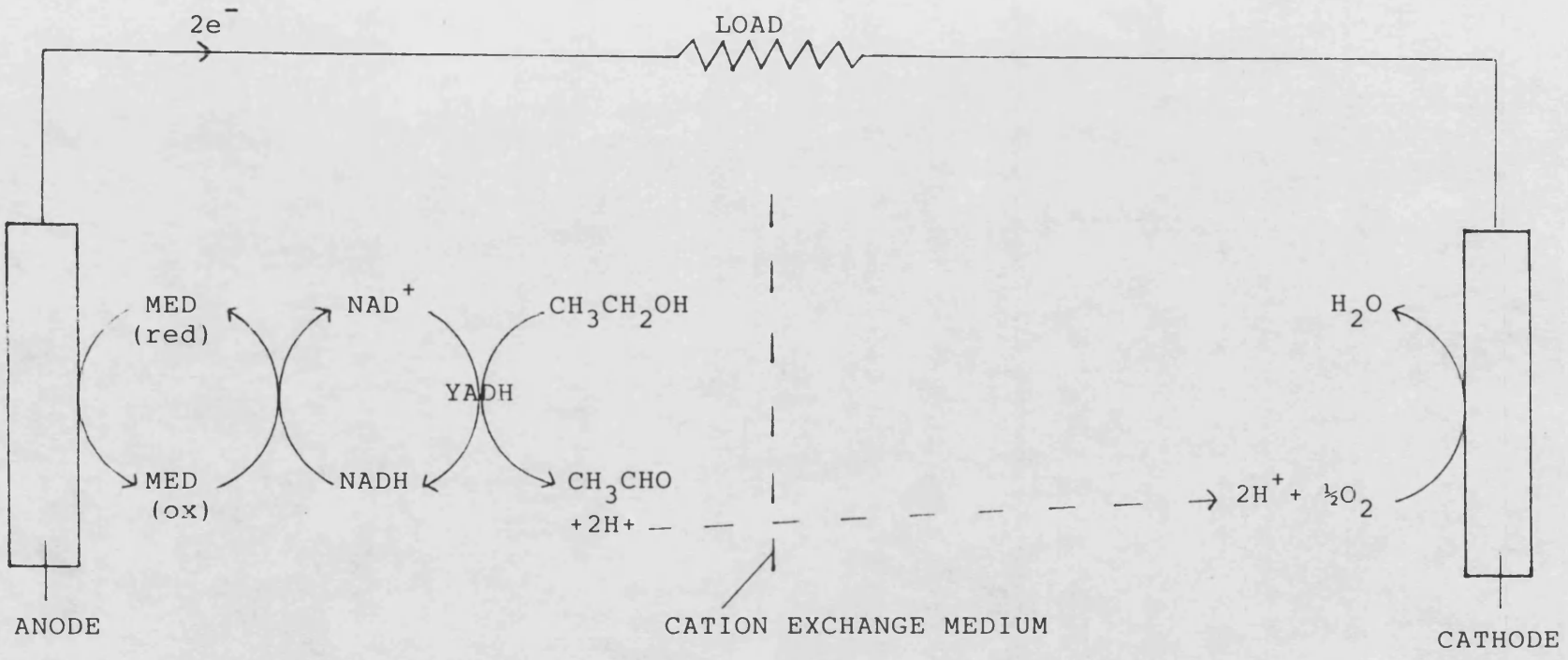


FIGURE 3.1. SCHEMATIC REPRESENTATION OF A BIOFUEL CELL RUNNING ON ETHANOL

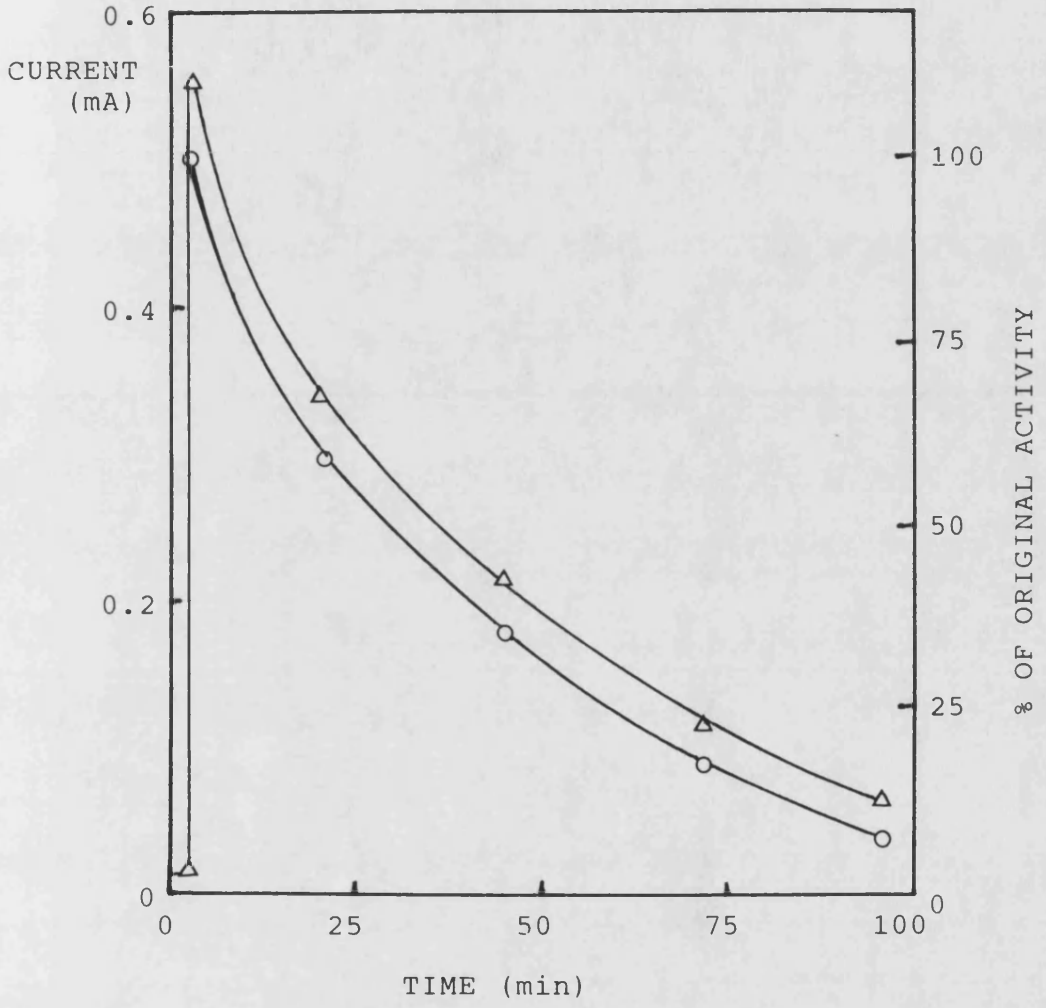


FIGURE3.2. CURRENT (△——△) AND ENZYME ACTIVITY (○——○) DECAY FOR CELL WITH YADH IN FREE SOLUTION.

(see text for experimental conditions)

a $t_{\frac{1}{2}}$ of 55 minutes. The YADH configurations appeared to be more suitable for use in the fuel cell than those with alcohol oxidase, but still did not give satisfactory performance. Enzyme immobilisation, though, appeared to be a promising approach for solving the problems of enzyme and current stability. Therefore, another method of immobilisation - the entrapment of enzyme within a polymeric gel set around the carbon cloth - was attempted.

3.1.3 Enzyme immobilisation in Polymeric Gels

(i) Polyacrylamide Gel (Hicks and Updike, 1966)

These gels are commonly prepared by mixing acrylamide (monomer) and N,N-methylene-bis-acrylamide (cross linking reagent) then catalysing the photopolymerisation with either riboflavin and potassium persulphate, or ultra-violet light under anaerobic conditions since oxygen inhibits the copolymerisation.

Unfortunately it was not possible to polymerise such a gel of sufficient flexibility and strength around the carbon cloth. Various concentrations of the gel were tried, catalysing the photopolymerisation with both of the above methods, and even polymerisation under vacuum was attempted but with no success. The cause of failure was most probably due to oxygen entrapped in threads of the cloth.

(ii) Starch Gel

The method for the production of a starch gel

was adapted from that of Bauman et al. (1965):-
2g of hydrolysed starch was placed into 5ml of 0.06M pyrophosphate buffer pH8.5 and the cold slurry poured into a boiling mixture of 13ml of buffer and 2ml glycerine. The resulting mixture was boiled until a clear solution was obtained, after which it was covered and allowed to cool to 45°C. In another beaker 80 units of YADH were dissolved in 2.5ml of buffer and this solution was poured onto the starch solution at 45°C. The beaker was washed with a further 2.5ml of buffer making a total volume of 25ml. The enzyme/starch solution was then gently stirred for 10 seconds and immediately poured over the carbon cloth electrode in a shallow dish. The dish was then left overnight at 4°C to set the gel. After setting, the electrode plus a layer of surrounding gel was cut from the dish and placed in the anodic compartment of the fuel cell along with electrolyte, ethanol, NAD and PES as in 3.1.2.

A maximum current of 200μA was produced with $t_{\frac{1}{2}}$ of 1½ hours. Considering that there were approximately 20 units of enzyme present in the gel attached to the electrode - only that amount of gel would be reasonably accommodated in the anodic compartment - the current output was very low. This could have been due to (i) denaturation of the enzyme when it was mixed with the starch solution at 45°C, or (ii) the gel itself inhibiting transport processes in the cell, particularly the transport of reduced mediator to the electrode surface. The current output was however more stable than previously achieved, suggesting that such immobilisation could

be an effective way of enhancing current stability. Unfortunately the starch gel was not robust enough for use in the fuel cell as it slowly disintegrated during a cell run due to the stirring and gassing present.

(iii) Carrageenan Cell

Carrageenan is a polysaccharide isolated from seaweed that can be used to immobilise many types of enzymes and microbial cells under mild conditions using appropriate gel inducing agents. The method used to produce such gels was adapted from that of Wada et al. (1979):-

A 2% solution of Kappa carrageenan was prepared using 5ml pyrophosphate buffer, pH8.5, and allowed to cool to 40°C. 20 units of YADH (in 0.1ml buffer) were added and the solution stirred for 30 seconds. The mixture was then pipetted (using a warmed Gilson tip) onto both sides of the carbon cloth forming a thin -2-3mm - flexible layer around the cloth. The gel was totally set after 5 minutes at room temperature with approximately 3ml of gel (12 units of YADH) around the electrode. The electrode was then placed in the cell along with the electrolyte, ethanol, NAD and PES as in 3.1.2.

The maximum current produced was 373μA with a $t_{\frac{1}{2}}$ of 2 hours. The current output of the cell was an improvement on that using a starch gel but not as good as that obtained with the enzyme in free solution - again most probably due to enzyme denaturation (assays of the gel showed that roughly one quarter of the original activity was retained after immobilisation) and the

inhibition of transport processes.

The gel itself was more stable than the starch gel, but still some disintegration had occured by the end of the run. At this point samples of the gel were assayed and no enzyme activity could be detected. Hence enzyme immobilisation around the electrode, while enhancing the stability of the current output to some extent, did not solve the problem of loss of enzyme activity.

3.1.4 The Effect of Alternative Mediators on Fuel Cell Performance

(i) N,N,N',N'-Tetramethyl-4-phenylenediamine(TMPD)

TMPD appeared to be the most suitable candidate as Davis et al.(1983) had reported an enhancement of current output stability with this mediator compared to PES in their fuel cell. The performance of TMPD was compared to that of PES with YADH in free solution. Thus, the anode compartment contained 9ml of electrolyte-0.06M pyrophosphate buffer pH8.5, 100mM ethanol, 0.5mM NAD, 2.5mM TMPD and 6 units of YADH.

A lower current was produced - 260μA - compared to that with PES and the stability was greatly reduced, $t_{\frac{1}{2}}$ = 8 minutes. Similar results were obtained using TMPD as the mediator with alcohol oxidase; therefore it was concluded that TMPD was unsuitable for these fuel cell configurations.

(ii) Ferrocenemonocarboxylic acid (FMCA)

Ferrocenes were suggested as possible mediators

at enzyme electrodes by Turner (1983). Cass et al. (1984) examined the use of several ferrocene derivatives and from these findings and due to its solubility, FMCA was chosen. The cell was set up as above, but with 2.5mM FMCA instead of TMPD. No significant current was obtained with either enzyme. A likely explanation of this is the electron withdrawing effect of the carboxyl group which would make the compound relatively hard to oxidise.

(iii) Hydroxyethylferrocene (HEF)

HEF was chosen because it would hopefully be more easily oxidised than FMCA and was reported to be soluble, and stable in the oxidised form (Szentrimay et al, 1977). The cell was set up as in 3.1.4(i) but with 2.5mM HEF instead of TMPD. A lower current -215μA- was produced than with TMPD, with similar poor stability, $t_{\frac{1}{2}}$ = 10 minutes.

A slightly better result was obtained with alcohol oxidase. The anodic compartment contained 9ml of electrolyte - 0.2M H_3BO_3, 0.05M NH_4Cl buffer pH8.0, 275mM methanol - required to dissolve the HEF - 2.5mM HEF and 5mg alcohol oxidase. The resultant current - 275μA - was similar to that obtained with TMPD and while the output was more stable than any previously obtained with alcohol oxidase in free solution ($t_{\frac{1}{2}}$ = 26 minutes) it was concluded that HEF was also unsuitable for these fuel cell configurations.

The use of these alternative mediators did not solve the problem of current instability; therefore, it

was concluded that loss of current might not be a mediator effect,but possibly due to some electrical phenomena acting on the enzyme at the electrode surface while the cell was under load, thus denaturing the enzyme. To test this hypothesis the enzyme was kept physically separate from the electrode in the cell.

3.1.5 Physical Separation of YADH from Electrode.

(i) YADH Immobilised in Agarose Beads

This commercial preparation was found to have an activity of 60 units/g solid at room temperature, pH8.5. A total of 70mg, equivalent to 4.2 units of enzyme, was suspended in distilled water, spun down, and washed several times with buffer before use in the cell. The anodic compartment contained 9ml of electrolyte - 0.06M pyrophosphate buffer pH8.5, 100mM ethanol, 0.5M NAD, 2.5mM PES and 4.2 units of YADH attached to beaded agarose. The current produced was 490μA indicating little inhibition of transport rates compared with the earlier gel immobilisations. More importantly, the stability of the current output was $t_{½}$ = 2 hours 35 minutes (Figure 3.3). This supported the view that physical separation of enzyme and electrode would produce greater stability, but when the beads were recovered from the cell at the end of a run it was found that they retained only 2% of their original activity. Agarose beads incubated in buffer for 4 hours at room temperature with stirring were found to lose 25% of their enzyme activity.

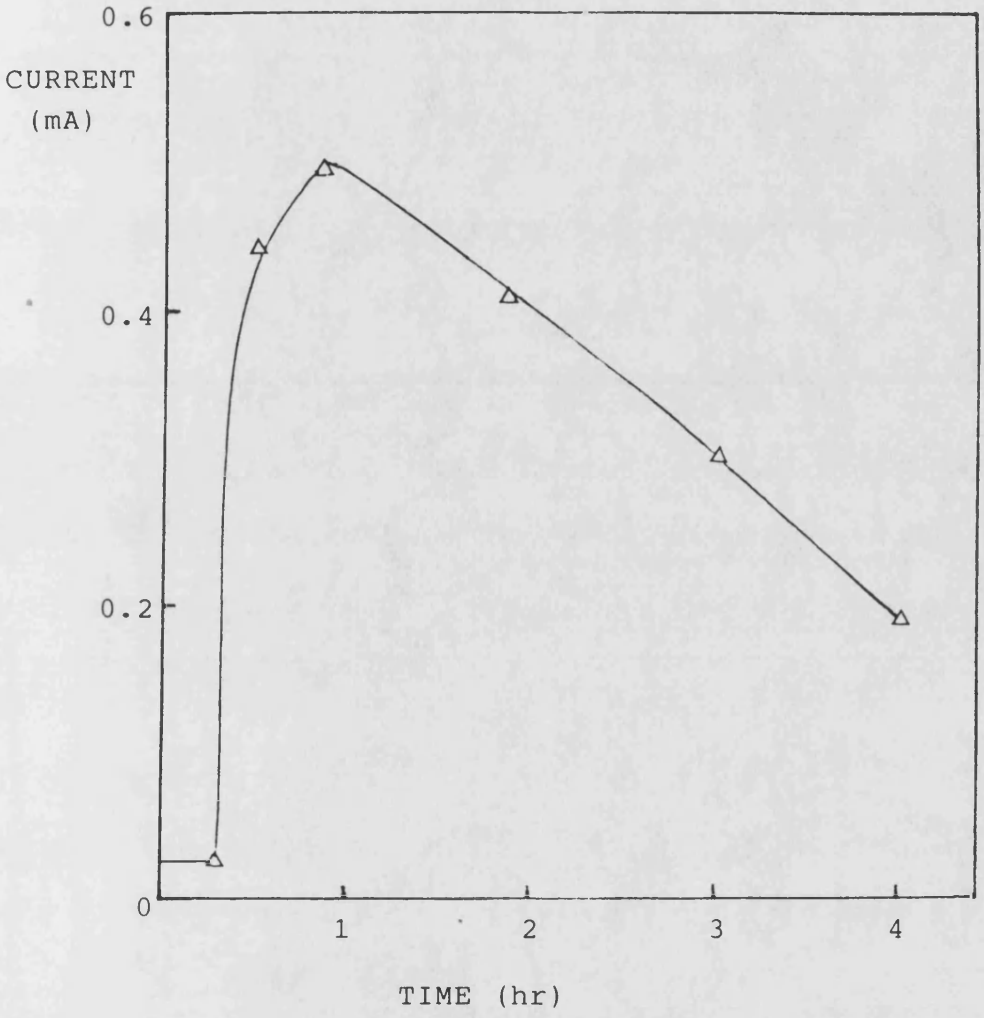


FIGURE 3.3. CURRENT DECAY FOR CELL WITH YADH IMMOBILISED IN AGAROSE BEADS

(see text for experimental conditions)

The above results could be due to the attainment of only a partial physical separation as the agarose beads would still come into contact with the electrode surface through the mixing of the contents in the compartments, therefore immobilisation methods resulting in a total physical separation of enzyme and electrode were attempted.

(ii) YADH immobilised in Carrageenan Gel on the surface of the Cation-exchange Membrane

A 2.25% solution of Kappa Carrageenan was prepared using 0.06M pyrophosphate buffer, pH8.5. The solution was cooled to 34°C, then 20 units of YADH were added and the mixture stirred for 30 seconds before being pipetted onto the anodic side of the cation-exchange membrane in the cell. The gel was then left at 4°C, in buffer, for 30 minutes prior to use. Electrolyte, ethanol, NAD and PES were added to the anodic compartment as before.

A maximum current of 128μA was produced with a $t_{\frac{1}{2}}$ of 33 minutes. It was thought that the low current obtained could have been due to the gel blocking hydrogen ion transport across the membrane, but the addition of enzyme in free solution to the anodic compartment in the above configuration produced higher currents (the addition of 12 units produced 760μA), showing that this was not a limiting factor. The more likely cause of the low current was probably an increase in the time taken for the mediator to diffuse between the enzyme and the electrode. It was noticeable that a proportion of the

mediator had become entrapped in the gel by the end of a cell run.

As with the carrageenan gelled around the electrode, no enzyme activity was detectable in the gel at the end of a cell run (gel incubated for 2 hours in buffer at room temperature with stirring was found to lose 8% of its enzyme activity). This could have been due to (i) bleeding of the enzyme from the gel, or (ii) a change in the pH - measurements of the pH in both compartments showed very little change after a cell run, but it is not known what changes may have occured in the microenvironment immediately surrounding the enzyme. Poor diffusion through the gel would in effect accelerate any local pH changes and this could account for the poor output stability.

An alternative method of immobilisation to achieve a total separation of the enzyme from the electrode would be to attach the enzyme to a nylon mesh positioned adjacent to the cation-exchange membrane. This approach would hopefully solve the diffusional problems encountered with the gel.

(iii) Immobilisation of YADH on Nylon Mesh

The procedure used was adapted from that of Salleh (1982) for the immobilisation of glucose oxidase, and that of Salleh and Ledingham (1981) for the immobilisation of urinase to a nylon tube.

The nylon mesh - 4cm x 2.5cm - was incubated in 18.5% (w/v) glutaraldehyde in 0.5M borate buffer, pH9.0, for

15 minutes at 90°C. The mesh was washed with 0.5M sodium chloride, then incubated in 10% (w/v in distilled water) polyethyleneimine at room temperature for 2 hours. The mesh was then washed with distilled water. The derivatised nylon mesh was reactivated for 10 minutes in 5% glutaraldehyde in 0.2M borate buffer, pH8.5,at room temperature. The mesh was washed with 0.1M sodium pyrophosphate buffer, pH8.5 and then immediately immersed in 10ml of enzyme solution (1mg/ml in 0.1M sodium pyrophosphate buffer, pH8.5) and incubated for 3 hours at 4°C.

After coupling the YADH-mesh was washed with 0.06M sodium pyrophosphate buffer, pH8.5 and stored at 4°C in the same buffer. The mesh was assayed for enzyme activity by measuring the rate of NADH production spectrophotometrically and was found to have retained 0.5 units. The mesh was attached to the membrane frame in the anodic compartment of the fuel cell with silicone rubber and electrolyte, ethanol, NAD and PES added to the compartment as before.

The small amount of activity present meant that only a low current of 90μA was produced. The stability ($t_{½}$ = 63 minutes) was an improvement on that obtained with carrageenan next to the membrane, but again no enzyme activity could be detected in the mesh at the end of a run. Nylon mesh incubated in buffer for 90 minutes at room temperature with stirring was found to lose 10% of its enzyme activity.

The low activity retained on the mesh obviously

severely limited the performance of this configuration. Salleh (1982) had stated that the final activity retained on immobilisation may be influenced by the suitability of the particular environment created for the particular enzyme molecule. Thus it could be the case that YADH was not suited to this method of immobilisation and so the next logical step was to try glucose oxidase (which had been shown to be suited to nylon immobilisation and to use in fuel cells) to investigate further the problems of enzyme instability and immobilisation away from the electrode (Section 3.2).

3.1.6 Alcohol Oxidase Configurations under Potentiostatic Control

With glucose oxidase configurations the potentiostat proved to bea useful tool in determining the cause of poor fuel cell performance (section 3.3) and it was therefore applied to the alcohol oxidase system.

The electrode chambers were filled with 13ml 0.25M potassium hydrogen phosphate buffer, pH8.0. HEF was then added giving a final concentration of 2.5mM in the working chamber, the potential was poised at +280mV and the system allowed to come to Nernstian equilibrium. 5mg of alcohol oxidase was added and a peak of 12mA was produced which decayed in 5 minutes. Methanol was added - to give a concentration of 5mM - but with no response. Samples of the chamber showed that there was still over 60% of the original activity present at this point; therefore permanent loss of activity could not be the explanation for the lack of a response to methanol.

Similar results were obtained using 2.5mM PES and DCPIP in the cell - in each case the alcohol oxidase reduced the mediator resulting in a peak of current, then the cell gave no response to the addition of methanol.

The substrate independent reduction of mediator could be observed in the spectrophotometer - oxidised HEF gives a blue solution (λmax 630nm) compared to the pale yellow colour of reduced HEF. e.g. A 2.5mM solution of oxidised HEF in 0.25M phosphate buffer, pH8.0, gave an absorbance reading of 0.78. The addition of alcohol oxidase immediately reduced the reading to 0.39. In comparison glucose oxidase only reduced the mediator in the presence of substrate.

It was clear that the currents produced in the earlier fuel cell experiments with alcohol oxidase were due to reaction with the mediator and not methanol. It must be noted that in the early experiments with alcohol oxidase the mistake was made of adding methanol to the fuel cell prior to PES and hence the unusual enzyme-mediator interaction was not discovered.

Table 3.1 Summary of Configurations with Alcohol as Fuel

Congiguration	Imax (μA)	$t_{\frac{1}{2}}$ (hrs.mins)
AOD, PES	475	0.10
AOD ABSORBED ON CLOTH, PES	750	0.27
YADH, PES	570	0.40
YADH, ABSORBED ON CLOTH, PES	550	0.55
POLYACRYLAMIDE GEL	UNSUITABLE	
YADH IN STARCH GEL, PES	200	1.30
YADH IN CARRAGEENAN GEL, PES	375	2.00
YADH/AOD, TMPD	260	0.08
YADH, FMCA	0	-
YADH, HEF	215	0.10
AOD, HEF	275	0.26
YADH-AGAROSE BEADS, PES	490	2.35
YADH IN CARRAGEENAN AT MEMBRANE, PES	128	0.33
YADH-NYLON MESH, PES	90	1.03
AOD IN POTENTIOSTAT, HEF	ENZYME-MEDIATOR	
" " " , PES	REACTION	
" " " , DCPIP	"	

3.2 Configurations with Glucose as Fuel

The basic configuration of a biofuel cell utilising glucose is shown in Figure 3.4.

3.2.1 Mediator Optimisation

Before any immobilisation of glucose oxidase was attempted a number of mediators were screened for their suitability in the fuel cell using the enzyme in free solution. For these studies the anodic compartment contained 9ml electrolyte - 0.1M KH_2PO_4 buffer, pH8.0 - 50mM glucose, 0.4mg GOD and 2.5mM mediator. Oxygen-free nitrogen was passed through the compartment in all cases except for that using HEF where the cell's output was insensitive to the presence of oxygen. The cathodic compartment contained 9ml electrolyte - 0.1M KH_2PO_4, 20mM KCl buffer, pH2.7, and was gassed with oxygen. Table 3.2 shows the maximum current and $t_{\frac{1}{2}}$ obtained with each mediator.

Generally the performances were much more encouraging than those with alcohol as a fuel, with, in certain cases, much higher currents and more stable outputs obtained. Benzoquinone gave the highest current and appeared the most promising mediator if the problem of stability could be overcome. Hydroxyethylferrocene also appeared a useful mediator giving a lower current but with greater stability.

The screening showed that there was little relationship between the formal potential of a mediator

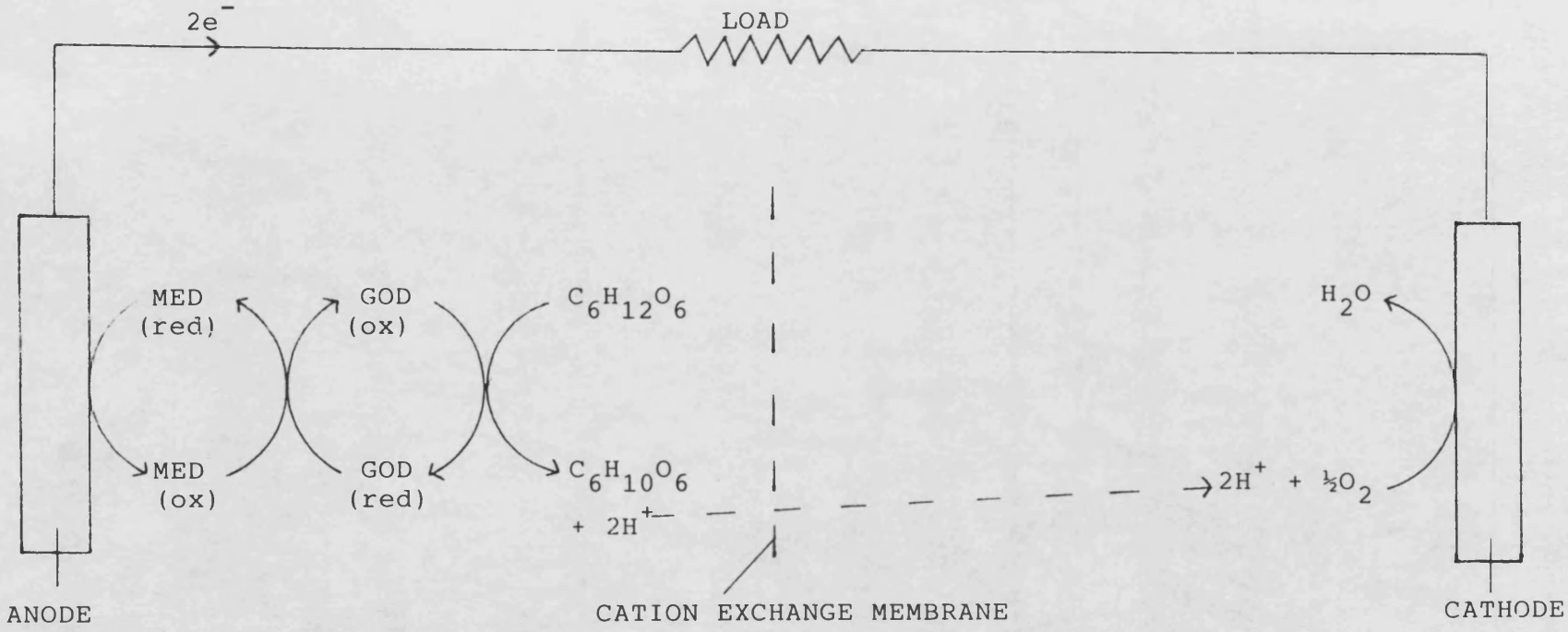


FIGURE 3.4. SCHEMATIC REPRESENTATION OF A BIOFUEL CELL RUNNING ON GLUCOSE

Table 3.2 Performance of GOD Fuel Cell with Different Mediators

(See text for experimental conditions)

Mediator	E°'(vsN.H.E)*	Maximum current (μA)	$t_{\frac{1}{2}}$ (hrs.mins)
Benzoquinone	0.280	1440	1.45
Hydroxyethyl ferrocene	0.402	820	5.00
Toluidine Blue-O	0.027	520	4.00
Methylene Blue	0.011	350	2.15
Phenozine Ethosulphate	0.055	250	4.40
Basic Blue 24	-0.021	180	1.30
Dichlorophenol-indolphenol	0.217	65	4.00
Methyl-Viologen	-0.440	negligible	-
Thionine	0.060	negligible	-

*E°' is the conditional or formal potential and may be regarded as a standard potential for a particular medium in which the activity coefficients are independent (or approximately so) of the reactant concentrations.

and the fuel cell's output and stability. This raised the question why the mediators were producing different performances. Some mediators might just not be suited to electron transfer with this particular enzyme, but there could be other influencing factors (this will be discussed more fully in section 4.2). The carbon cloth electrodes are known to be highly absorbent, especially to organic molecules such as the above mediators. It seemed possible, therefore, that a progressive irreversible absorption of the mediators onto the cloth could result in increased ohmic polarization due to a layering effect. If this was the case then the different outputs and stabilities could be accounted for by differing rates and patterns of absorption by different mediators.

To investigate the above hypothesis mediator was pre-absorbed onto the carbon cloth before use in the cell. Hence if the electrode was indeed poisoned by the mediator only a low current would be observed. Benzoquinone was chosen for the test as it had produced the highest current in the cell. The cloth was incubated in 125mM solution of the mediator overnight at room temperature, then rinsed thoroughly with MilliQ water before use in the cell. The cell conditions and constituents were identical to the previous experiments except for the absence of mediator in free solution. The maximum current produced was 1800μA with a $t_{½}$ of 1½ hours.

The pattern of decay was very similar to that

using mediator in solution, therfore it was clear that progressive absorption of the mediator was not the cause of the unstable output.

Another possibility was that instead of mediator absorption, enzyme absorption/interaction at the electrode was responsible for the current decay. In the alcohol fuel cell the most promising approach to deal with this problem was to try to obtain a total separation of enzyme and electrode. This approach was therefore followed in the glucose fuel cell by immobilising the glucose oxidase onto a nylon mesh which would then be positioned adjacent to the cation-exchange membrane in the anodic compartment of the fuel cell.

3.2.2 Glucose Oxidase Immobilised on Nylon

Nybolt 63 Normal nylon mesh was used for the initial immobilisation attempts. The methods used were adapted from those of Salleh and Ledingham (1981), Salleh (1982) and Inman and Hornby (1972 and 1974). These covalent methods of attachment, although successful, did not result in a sufficient amount of active enzyme immobilised on the nylon for use in a fuel cell, due mainly to the low surface area of the mesh.

A nylon membrane thought to be capable of carrying larger amount of enzyme was therefore employed - Pall Biodyne A. This is a supported nylon 66 membrane with amine and carboxyl active surface groups in a 1:1 ratio for absorptive binding. The most effective immobilisation to this membrane was obtained simply by dotting the

enzyme solution onto the membrane and then leaving it to dry in the cold room. The membrane was cut to the same size as the cation-exchange membrane. The GOD solution (0.4ml buffer containing 1.6mg = 48 units) was then lightly dotted onto that area of the membrane which would be in contact with the anodic solution. The membrane was left to dry thoroughly for 48 hours at 4°C. At the end of this period the nylon membrane was carefully sealed in the membrane frame, on the anodic side of the cation-exchange membrane, using silicone rubber and the frame then clamped in the fuel cell body. The fuel cell was set up as previously: the anodic compartment contained 9mls electrolyte - 0.1M KH_2PO_4 buffer, pH8.0, 2.5mM Benzoquinone, 50mM glucose and was gassed with oxygen-free nitrogen. The cathodic compartment contained 9ml electrolyte - 0.1M KH_2PO_4, 20mM KCl buffer pH2.5 and was gassed with oxygen.

The maximum current obtained was 1100μA with a $t_{\frac{1}{2}}$ of $1\frac{1}{4}$ hours. The current could have been limited by the amount of active enzyme left on the membrane after the "dry" incubation or by concentration polarisation i.e. the distance the mediator had to travel to shuttle electrons between the enzyme and the electrode. More importantly there was no increase in the stability of the output, suggesting that an enzyme-electrode interaction was not responsible for current decay. Unfortunately, though, some enzyme (approximately 3 units) had leaked form the membrane; a more reliable immobilisation was therefore needed to confirm the above conclusion.

A thin protein film was constructed (following

the method of Thomas and Brcun (1976) by mixing 0.4mg God, 0.25ml BSA solution (60mg/ml in buffer) and 70µl glutaraldehyde (2.5% in distilled water). When the viscosity of the mixture began to increase the solution was spread on a perfectly plane glass plate, inside the limits of a rectangle drawn with a glass pencil, taking care to avoid air bubbles. The plate was covered and left at 4°C overnight. A yellowish membrane was produced that was removed from the plate by soaking in distilled water for 1 hour, in 0.1M lysine for 45 minutes, and then in distilled water again for 15 minutes. The membrane was checked to exclude enzyme leaking by immersing it in buffer and assaying the solution for enzyme activity and then positioned adjacent to the cation-exchange membrane in the fuel cell.

A maximum current of 1000µA was produced with a $t_{\frac{1}{2}}$ of 2 hours 15 minutes. After 4 hours 30 minutes the current had decreased to 25% of the maximum. At this point the film was removed from the cell and was found to contain over 50% of the original activity (hence there had been some inactivation of the immobilised enzyme). The $t_{\frac{1}{2}}$ was only slightly higher than that with enzyme in free solution showing that an enzyme-electrode interaction was not a major influence on the stability of the output. The retention of enzyme activity in the film also suggested that, in contrast to the alcohol configurations, the stability of the output was not limited by the stability of the enzyme.

The relationship between enzyme activity in free solution and the cell's output was then investigated,

in this case the activity could be measured throughout the cell run and not just at the end.

3.2.3 Measurement of GOD Activity in Free Solution

The anodic compartment contained 9ml electrolyte-0.1M KH_2PO_4 buffer pH8.0, 0.4mg GOD, 2.5mM benzoquinone, 50mM glucose and was gassed with oxygen-free nitrogen. Aliquots of the solution were removed and assayed throughout the run for enzyme activity. The cathodic compartment contained 9ml electrolyte-0.1M KH_2PO_4, 20mM KCl buffer pH2.7 and was gassed with oxygen.

Two controls were constructed and monitored for enzymic activity

(i) containing the anodic solution with gassing and stirring in a cell under open circuit conditions.

(ii) as (i) but with no electrode present.

Figure 3.5 shows the resultant output and corresponding activity measurements. Control (ii) showed glucose oxidase to be extremely stable under experimental biofuel cell conditions. In the presence of the unconnected carbon cloth electrode the activity in solution dropped to just over 50% in 150 minutes then remained constant, indicating absorption of the enzyme onto the electrode surface. During the cell run the enzyme activity dropped to 20% of the original value in 20 minutes and then remained between 10 and 20% for the remainder of the run. The drop in enzymic activity in solution did not correlate with the current decay - it is possible that more of the enzyme had absorbed onto the electrode at

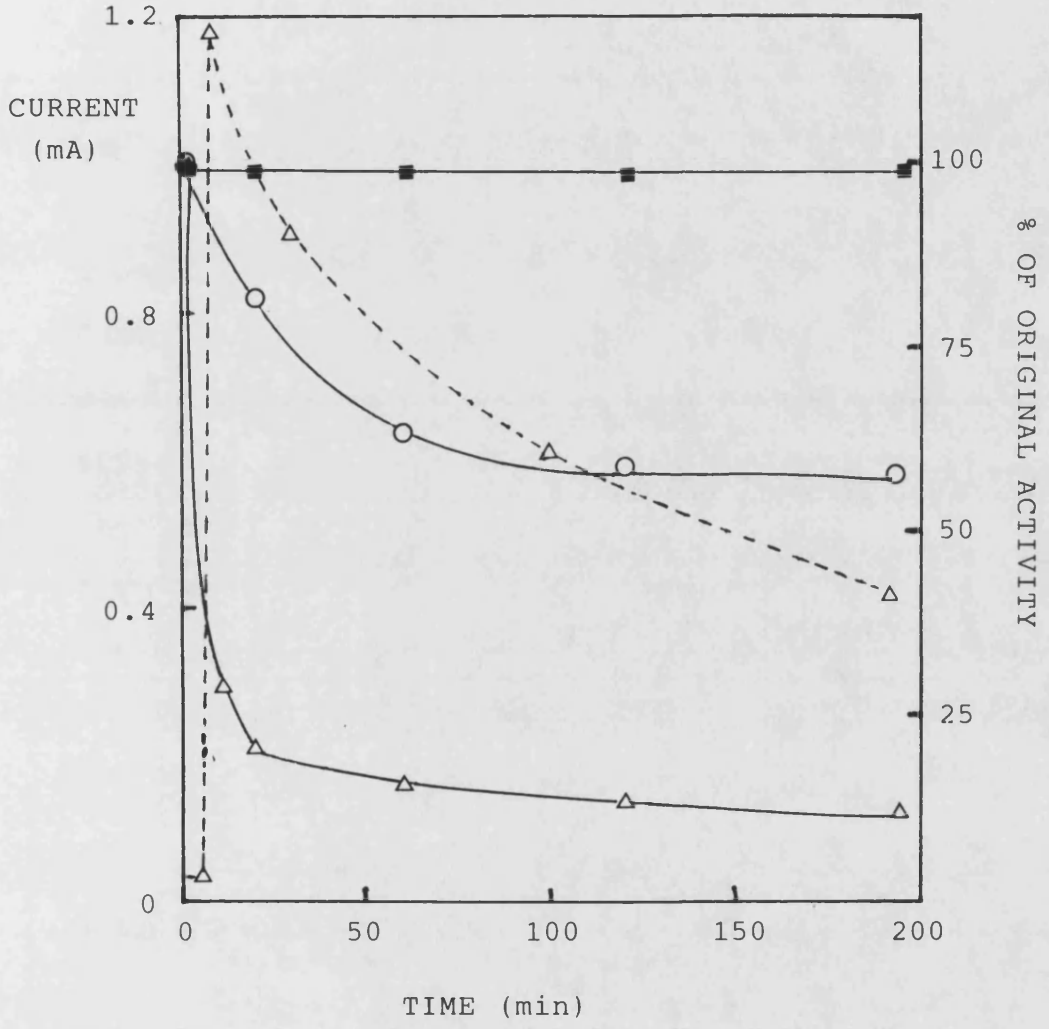


FIGURE 3.5. CURRENT DECAY (△--△) IN GOD FUEL CELL WITH BENZOQUINONE AS MEDIATOR. ENZYMIC ACTIVITY WAS MONITORED IN THE FUEL CELL UNDER LOAD (△——△); UNDER OPEN CIRCUIT CONDITIONS (○——○) AND WITH NO ELECTRODE PRESENT (■——■).

(see text for experimental conditions)

a greater rate than the control, and had seemingly retained activity. If this was the case then the enzyme had then been strongly attracted to the anode but remained an effective catalyst indicating that some other factor was responsible for the current decay.

The stability of the carbon cloth electrodes was subsequently questioned. The structure and arrangement of the carbon cloths in the cell were not suited to ideal mixing and this could lead to the formation of "dead zones" during a cell run causing increased concentration polarisation. In an attempt to avoid this effect and to check for any other limitations on the fuel cell's performance caused by the carbon cloth electrodes, platinum gauze electrodes were constructed for use in the fuel cell.

3.2.4 Platinum Electrodes in the Glucose Fuel Cell

The anodic compartment contained 11.5ml electrolyte- 0.1M KH_2PO_4 buffer pH8.0, 2.5mM Benzoquinone, 0.4mg GOD and 50mM glucose. The cathodic compartment contained 11.5ml electrolyte- 0.1M KH_2PO_4, 20mMKCl buffer, pH2.7. The gassing and stirring were identical to other configurations.

The maximum current obtained was 770μA with a $t_{½}$ of 20 minutes. The surface areas of the platinum electrodes were smaller than that of the carbon cloth electrodes; therefore, the current output was reduced. The poor stability was surprising and without an obvious explanation except perhaps that the benoquinone was

not reacting reversibly at the electrode. Laane et al. (1984) had used a similar configuration in a fuel cell but using the mediator DCPIP, and had obtained stable cuurents of 330μA for at least 3 hours. DCPIP (0.1mM) was therefore used in the fuel cell instead of benzoquinone to attempt to reach a stable configuration. The maximum current obtained was 290μA with a $t_{\frac{1}{2}}$ of 35 minutes.

The one remaining major difference between the above experimental fuel cell and that of Laane was the presence of a cation-exchange membrane as opposed to a salt bridge to allow the passage of hydrogen ions from the anode to the cathode. It was possible that the membrane could have been short circuiting the cell, i.e., substances that should have been localised in the compartments had equilibrated across the membrane e.g. oxygen could have been reacting directly with the reduced mediator, or that the pores in the membrane were becoming blocked.

The membrane and membrane frame were, therefore, replaced in the cell by a solid block of perspex containing three holes of 6mm diameter in the centre of the block. The holes were filled with 3M potassium chloride in 1·5% agar-agar to produce the salt bridge.

The experiment with DCPIP was repeated and produced a maximum current of 120μA with a $t_{\frac{1}{2}}$ of 40 minutes. Thus, the salt bridge did not cause any improvement in the cell's performance. Hence it appeared that the platinum gauze electrodes were less suitable for use in the experimental fuel cell than the carbon cloth electrodes.

It was clear that further experiments with the fuel cell would be futile unless the fundamental problem of stability could be approached. A modified cell was therefore constructed suitable for use under the control of a potentiostat, figure 2.3.

3.3 Configurations under Potentiostatic Control

A potentiostat holds the potential of a working electrode constant relative to a reference electrode, making it easier to determine what is limiting the current. A chart recorder was used to measure the current flowing between the working electrode and the counter electrode; for each addition of glucose the area under the current vs. time trace was measured to give the number of coulombs passed. This then gave a measure of the system's efficiency in converting glucose to electricity, as the theoretical coulombic yield could be calculated from the amount of glucose added (assuming the release of two electrons for every mole of glucose consumed).

3.3.1 Benzoquinone as Mediator

The working and counter compartments of the potentiostat cell each contained 10ml electrolyte - 0.1M KH_2PO_4 buffer pH8.0. Benzoquinone (to give a final concentration of 2.5mM) was added to the working compartment, the potential poised at +130mV (120mV positive of E°' for benzoquinone) and the system allowed to come to Nernstian equilibrium. GOD (0.4mg) was added followed by 0.1ml 1M glucose. Table 3.3 shows the current produced for successive additions of glucose.

With each addition the cell produced less current and took a longer time to utilise the glucose, indicative of a gradual poisoning of the electrodes. To see what effect this might have on the maintenance of a stable

current the experiment was repeated but instead of adding glucose in "batch" form giving peaks of current the glucose concentration was kept fairly constant by adding 20µl of 1M glucose every 5 minutes after the initial addition.

Table 3.3 Response of the cell to successive additions of glucose See text for experimental conditions.

Addition	Maximum Current (mA)	Peak length (hr.min.)	Coulombic Efficiency %
1	19.0	1.00	>90%
2	16.0	1.20	>90%
3	12.5	1.45	>90%

The resultant current peaked at 16.5mA and then was stable at 14mA for 30 minutes; but had decayed to 87% after 1 hour, and to 3% after 2 hours. At this point further additions of glucose and GOD produced no effect on the current, but the addition of benzoquinone did produce a small peak of 4mA.

The enzyme activity was monitored during the above configurations and was not found to change significantly. It seemed likely therefore that the drop in response could have been due to the benzoquinone; e.g. polymerisation may have occured. Hydroxyethylferrocene, which had earlier been shown to be a suitable mediator for the system, was then chosen to replace the benzoquinone.

3.3.2 Hydroxyethylferrocene as Mediator

The working and counter compartments of the cell each contained 10ml electrolyte - 0.1M KH_2PO_4 buffer pH8.0. HEF was added to the working compartment to give a concentration of 2.5mM, the potential poised at +280mV, and the system allowed to come to Nernstian equilibrium. GOD (0.4mg) was added followed by 0.1ml aliquots of 1M glucose. Table 3.4 shows the current produced for succesive additions of glucose.

Table 3.4 Response of the cell to successive additions of glucose See text for experimental conditions.

Addition	Maximum Current (mA)	Peak length (min)	Coulombic Efficiency %
1	22.0	42	>90
2	24.0	42	>90
3	26.0	48	>90
4	22.5	50	>90
5	17.0	56	70

Hydroxyethylferrocene provided an improved response from the cell, showing it to be a more suitable mediator for this system than benzoquinone, most probably due to its greater stability. A further addition of glucose, though only produced a current of 4mA, and pH measurements taken at this point showed that the pH in the working compartment had fallen to 4.2, while that in the counter compartment had risen to 11.6. These

readings were not wholly surprising since the number of hydrogen ions released from the oxidation of glucose could be enough to overcome the capacity of the buffer in the working compartment. Similarly the production of oxygen radicals and hydroxide ions at the counter electrode could account for the raised pH in the counter compartment.

A gradual reduction in pH in the working compartment could account for the increasing peak length and ultimately the reduced response from the cell. Glucose oxidase using oxygen as the oxidant is more active at pH4 than at pH8; but it is possible that with HEF as the oxidant the activity is reduced at lower pHs.

It was clear that, in the potentiostat at least, the pH would have to be controlled to produce a cell with consistent performance over a long time. The strength of the buffer was therefore increased to 0.25M, and the pH of the working compartment was adjusted to 8.0 after each peak by the addition of potassium hydroxide. The glucose concentration was also increased from 10mM to 100mM (i.e. well past saturation level) to ascertain whether the cell could maintain a steady current at peak level. Table 3.5 shows the current produced for successive additions of glucose (following page).

The adjustment of pH allowed the cell to give consistent responses over a twelve hour period which was a dramatic improvement on previous results, and the fact that the maximum current was maintained instead of decaying was also encouraging. The approaches found

successful in the potentiostated cell werethen applied to the fuel cell.

Table 3.5 Response of the cell to successive additions of glucose See text for experimental conditions

Addition	Maximum Current (mA)	Peak length (hrs.mins)	Coulombic Efficiency %	Working compartment pH at end of peak
1	29	2.45	>90	5.3
2	29	2.45	>90	6.0
3	25	3.00	>90	5.9
4	25	3.00	>90	5.6

For each addition the current was stable at the maximum for approximately 45 minutes before decaying steadily to zero.

3.4 Development and Evaluation of Glucose Fuel Cell

3.4.1 Development from potentiostatic configuration

The fuel cell was set up with identical constituents to those used in the potentiostat i.e. the anodic compartment contained 9ml of electrolyte- 0.25M KH_2PO_4 buffer pH8.0, 2.5mM HEF, 0.4mg GOD and 50mM glucose. The cathodic compartment contained 9ml electrolyte - 0.25M KH_2PO_4 buffer, pH8.0 and was gassed with oxygen.

A current of 130μA was produced that was stable for 16 hours. Additions of enzyme, mediator and substrate had no effect on the current. The only major difference between this and earlier fuel cell configurations was the cathodic buffer.

The experiment was therefore repeated using 0.25M HCl/KCl buffer pH2.0 as the cathodic electrolyte. Table 3.6 shows the current produced by the cell and the pH changes in the cell compartments - the values given in the table are those measured at the end of the current peaks.

The poor stability of the current output was wholly due to the lack of pH control in the cathodic compartment. The adjustment of the pH back to 2.0 with HCl was sufficient to bring the current up to maximum level. It was clear that the wrong choice of buffer had been made for the cathodic compartment - the HCl/KCl buffer was only effective over the pH range 1.0-2.0 and so did not control the pH above this level.

Table 3.6 Response of the cell to additions of glucose and HCl (to adjust the cathodic pH to 2.0)/ KOH (to adjust the anodic pH to 8.0).
See text for experimental conditions.

Addition	Maximum Current (mA)	$t_{\frac{1}{2}}$ (min)	pH at end of peak anode	pH at end of peak cathode	Remarks
Glucose	0.76	48	7.5	4.2	
HCl	1.10	100	7.2	2.2	cathode leaked
Cathodic buffer	0.80	40	7.2	6.2	decayed to 50μA overnight
Glucose	No peak	-	-	-	Added at $t_{\frac{1}{2}}$ in previous peak
HCl/KOH	1.03	60	7.2	5.0	

The experiment was again repeated, but with 0.25M sodium acetate/HCl buffer pH2.0 as the electrolyte. Table 3.7 shows the current produced by the cell under these conditions.

Table 3.7 Response of the cell to additions of glucose and HCl/KOH See text for experimental conditions

Addition	Maximum Current(mA)	$t_{\frac{1}{2}}$(min)	pH at end of peak anode	pH at end of peak cathode	Remarks
Glucose	0.92	9.30	7.0	4.3	
KOH/HCl (Glucose)	0.80	13.00	6.8	4.5	Glucose added 6h into peak stabilised current
KOH/HCl Glucose	0.74	10.00	7.0	4.8	
KOH/HCl	0.89	2.45	7.3	4.6	

The stability of the current output was now governed by three factors, (i) the level of glucose in the anodic compartment, (ii) the pH in the anodic compartment and (iii) the pH in the cathodic compartment. The latter was the most important factor, as adjustment of the cathodic pH was necessary for the complete oxidation of any one addition of glucose, e.g. after the current from the last addition of glucose had decayed until it was virtually constant at 200μA, the addition of KOH to the anodic compartment brought the current up to 370μA while the addition of HCl to the cathodic compartment brought the current up to 890μA and enabled the cell to utilise the remaining glucose.

The alteration of cathodic conditions had proved crucial to the performance of the fuel cell and it seemed likely that the cathodic reaction was still a limiting factor, which reduced the efficiency of the system. The electrochemical reduction of oxygen to water has been generally regarded as a difficult reaction to complete efficiently; consequently the reduction of potassium ferricyanide was chosen to provide a stable and relatively nonpolarisable cathodic reaction (Allen, 1972).

3.4.2 Potassium Ferricyanide as the Cathodic Reactant

The anodic compartment contained 9ml electrolyte-0.25M KH_2PO_4 buffer pH8.0, 2.5mM HEF, 0.4mg GOD and 50mM glucose. The cathodic compartment contained 9ml

electrolyte - 0.25M sodium acetate/HCl buffer pH2.0, 50mM potassium ferricyanide and was gassed with oxygen-free nitrogen.

After the first current peak had decayed the cell was relatively insensitive to pH adjustment in either compartment and a further addition of glucose. It was thought that most of the potassium ferricyanide had been reduced. This was confirmed by replacing the cathodic electrolyte with a fresh solution and obtaining maximum current again. Once the second batch of potassium ferricyanide had been exhausted, the cathodic electrolyte was replaced again, but with 250mM ferricyanide. This resulted in a 25% increase in the current output and a dramatic increase in the stability, as shown in Table 3.8.

Table 3.8 Response of the cell to additions of glucose, HCl/KOH and potassium ferricyanide.

See text for experimental conditions.

Addition	Maximum Current(mA)	$t_{\frac{1}{2}}$ (hr.min)	pH at end of peak	
			Anode	Cathode
Glucose	1.02	6.00	7.1	3.3
KOH/HCl/ glucose	0.46	0.30	-	-
50mM $K_3Fe(CN)_6$	1.10	7.00	7.3	3.6
KOH/HCl/ glucose	0.67	0.45	-	-
250mM $K_3Fe(CN)_6$	1.25	20.00	6.2	3.0
KOH/HCl/ glucose/ 250mM $K_3Fe(CN)_6$	1.05	16.00	6.2	3.2

The use of 50mM potassium ferricyanide gave no improvement on the experiments with oxygen as the cathodic reactant. The stronger solution on the other hand, gave the optimum performance achieved thus far, and appeared to be much less sensitive to the pH in the cathodic compartment (see section 3.5).

The output of the fuel cell was now stable enough for the measurement of further parameters of fuel cell performance such as the open circuit voltage, voltage-current plots, internal resistance and power output.

3.4.3 Evaluation of Fuel Cell Performance

Cell 1

The fuel cell was set up as in 3.4.2 with 250mM potassium ferricyanide in the cathodic compartment. To record the open circuit voltage glucose was added to the cell with just a voltmeter across the terminals. The external resistance (10Ω) and ammeter were then placed in the circuit and the current allowed to stabilise around 1mA. The external resistance was then varied between 0.5Ω and 10,000Ω, and the respective values of voltage and current measured. The voltage-current and power-current relationships are shown in Figure 3.6. Further parameters of fuel cell performance are given in Table 3.9.

Cell 2

In an attempt to lower the internal resistance and to improve the performance of the configuration

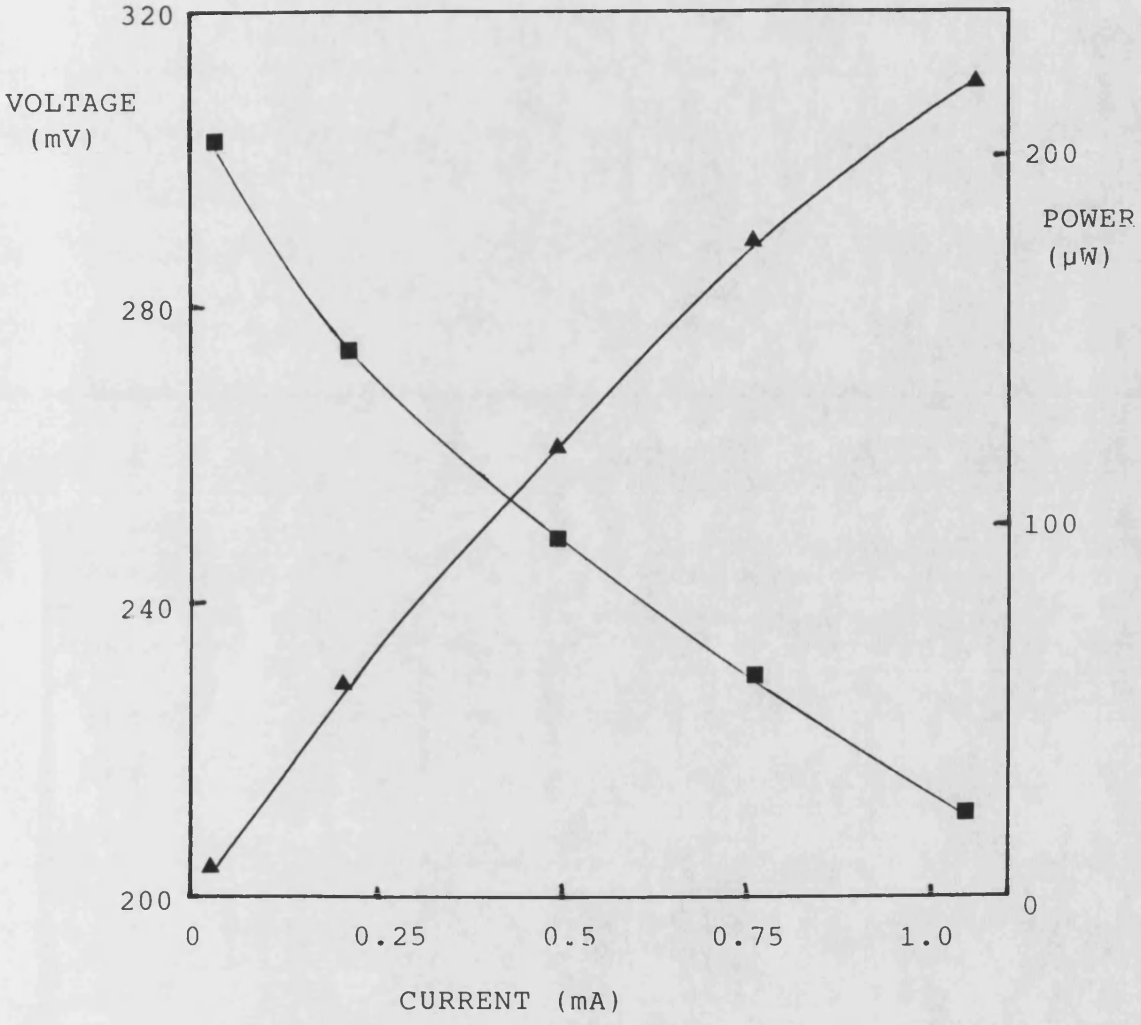


FIGURE 3.6. CELL 1: VOLTAGE-CURRENT RELATIONSHIP (■——■)
AND POWER-CURRENT RELATIONSHIP (▲——▲)

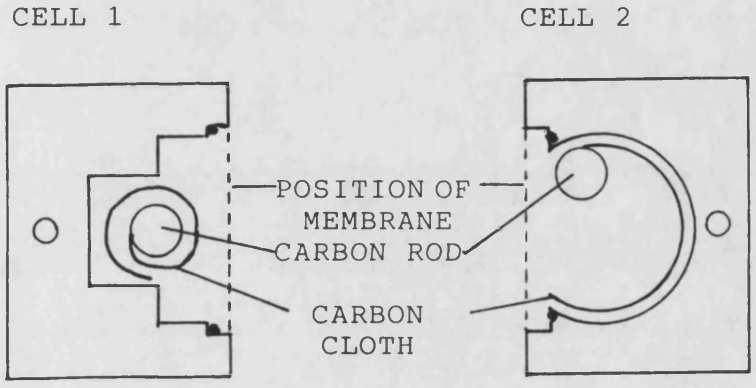
(see text for experimental conditions)

the design of the compartments was slightly modified, as shown in Figure 3.7. In cell 2 the carbon cloth could easily contour the circumference of the compartment, allowing more efficient mixing. The performance of cell 2 was then evaluated using the same experimental conditions as those used in cell 1. The voltage-current and power-current relationships are shown in Figure 3.8. Further parameters of fuel cell performance are shown alongside those of cell 1 in Table 3.9.

Table 3.9 A comparison of the various performance parameters for cells 1 and 2.

Parameter	Cell 1	Cell 2
Open circuit voltage(mV)	390	340
Internal resistance(Ω)	84	136
Max. current density(Am^{-2})	1.0	0.9
Max. power(μW)	220	159
Power density(mWm^{-2})	176	127
Voltage efficiency at 10Ω(%)	54.9	53.5

The modification of the cell did not increase the performance of the system, but resulted in an increase in the internal resistance and a reduction in the open circuit voltage and power output. The increased volume, coupled with the removal of the carbon cloths from the proximity of the cation exchange membrane (i.e. increasing the distance separating the electrodes) would have led to increased ohmic polarisation and hence the relatively



COMPARTMENT
VOLUME :- 9ml 13ml

FIGURE 3.7. COMPARTMENTAL DESIGN OF CELLS 1 AND 2.
(Drawn to scale)

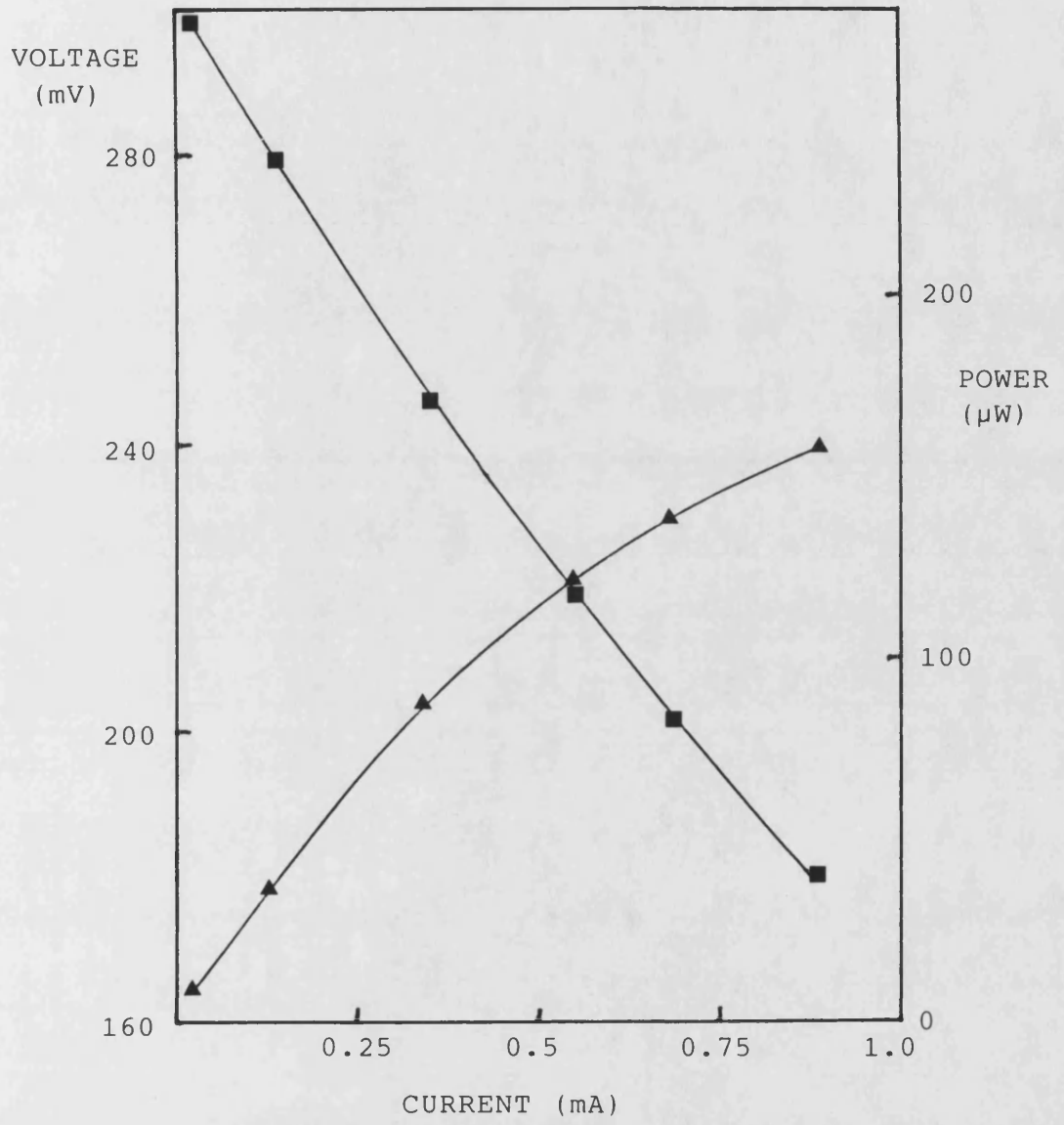


FIGURE 3.8. CELL 2: VOLTAGE-CURRENT RELATIONSHIP (■——■)
AND POWER-CURRENT RELATIONSHIP (▲——▲)

poor performance of the modified cell.

In comparison with other biofuel cells (e.g. Davis et al., 1983) the respective current and power densities obtained with this configuration appear most promising (see Section 4.5). However, a further improvement in stability was still required. The first approach was to assess the effect of the compartmental pH levels.

3.5 pH Effects in the Glucose/Ferricyanide Fuel Cell

3.5.1 The Anodic pH

Experiments with the potentiostated cell (Section 3.3) had shown the anodic reaction to be sensitive to a reduction in pH. With oxygen being reduced at the cathode, the pH in the anodic compartment was reduced from 8.0 to around 7.0 with a relatively slight effect on the current stability. With the reduction of ferricyanide at the cathode the anodic pH fell below 6.0 at the end of a peak and it seemed likely that this would have a more significant effect on the stability of the output.

A buffer of a higher pH was therfore used in the anodic compartment to attempt to improve the current stability. The compartment contained 9ml electrolyte- 0.25M sodium carbonate buffer, pH10.0, 2.5mM HEF, 0.4mg GOD and 50mM glucose. The cathodic compartment contained 9ml electrolyte - 0.25M sodium acetate/HCl buffer, pH2.0, 250mM potassium ferricyanide and was gassed with oxygen-free nitrogen.

The performance of this system was compared with that using 0.25M KH_2PO_4 buffer, pH8.0, as the anodic electrolyte (Table 3.10).

The sodium carbonate buffer kept the pH higher in the anodic compartment resulting in a more stable output than that achieved with the sodium acetate/HCl buffer. A further increase in pH would be deleterious to the enzyme and so the sodium carbonate buffer, pH10.0 was chosen as the anodic electrolyte for the remaining

experiments.

Table 3.10 Response of the cell to additions of glucose, HCl/KOH and ferricyanide; anodic pH at start of peaks = 8.0/10.0

See text for experimental conditions.

Addition	Maximum Current (mA)		t3/4 (hr.min)*		Anodic pH at end of peak	
	pH8	pH10	pH8	pH10	pH8	pH10
Glucose	1.03	1.00	6.00	11.00	6.3	7.4
Glucose/KOH/HCl /Ferricyanide	1.10	1.15	5.30	10.00	6.5	7.8
Glucose/KOH/HCl /Ferricyanide	1.07	1.05	5.45	10.20	6.2	7.7

*t3/4 = time taken for the current to decay to three quarters of the maximum value.

3.5.2 The Cathodic pH

The reduction of oxygen at the cathode had proved very pH sensitive so it was decided to see what effect, if any, the pH had on the reduction of ferricyanide, and on the performance of the cell.

The anodic compartment contained 9ml electrolyte-0.25M sodium carbonate buffer, pH10.0, 2.5mM HEF, 0.4mg GOD and 50mM glucose. The cathodic compartment contained 9ml electrolyte - 0.25M sodium acetate buffer pH2-4, or

0.25M potassium phosphate buffer pH6-8, or 0.25M sodium carbonate buffer pH10.0, 250mM potassium ferricyanide and was gassed with oxygen-free nitrogen.

Table 3.11 shows the effect of the differing cathodic electrolytes on fuel cell performance.

Table 3.11 Effect of differing cathodic electrolytes on fuel cell performance.

See text for experimental conditions

Cathodic pH	Maximum Current(mA)	t3/4 (hr.min)	pH at end of peak	
			Anode	Cathode
2.0	1.00	11.00	7.4	3.0
3.0	0.98	13.20	7.8	3.8
4.0	0.99	15.45	8.5	4.5
6.0	0.98	16.00	9.0	7.0
8.0	0.98	16.00	9.3	8.7
10.0	0.96	15.30	10.1	9.1

The maximum current produced by the fuel cell was independent of the cathodic pH. The stability of the current output was also independent of the cathodic pH above 4.0; the lower pH values resulted in the reduction of the anodic pH below 8.0 and hence the subsequent loss of stability.

In each case, at the end of the peak, the adjustment of pH and the addition of glucose only produced a small increase in the current. The replacement of the potassium ferricyanide solution though, brought the current back

up to its maximum value. The concentration of ferricyanide was therefore increased from 250mM to 1M.

3.5.3 Effect of Increased Ferricyanide Concentration on Output Stability

The anodic compartment contained 9ml electrolyte-0.25M sodium carbonate buffer, pH10.0, 2.5mM HEF, 0.4mg GOD and 50mM glucose. The cathodic compartment contained 9ml electrolyte - 0.25M KH_2PO_4 buffer pH6.0, 1M potassium ferricyanide and was gassed with oxygen-free nitrogen. Table 3.12 shows the performance of the cell after subsequent additions of the anodic and cathodic fuels.

Table 3.12 Response of the cell to additions of glucose, HCl/KOH and 1M ferricyanide

See text for experimental conditions.

Addition	Maximum Current(mA)	t3/4 (hr)	pH at end of peak Anode	Cathode
Glucose	1.14	18	9.0	6.4
KOH/Glucose	0.84	15	9.4	6.6
HCl/KOH/Glucose Ferricyanide	1.17	18	8.8	6.7
HCl/KOH/Glucose	0.78	14	9.5	6.5

The minor pH adjustments required at the end of the current peaks, as expected, had little or no effect on the current. The second addition of glucose produced a lowered current peak with reduced stability

indicating that at that stage the cathodic reaction was already beginning to limit the performance of the cell. This was indeed confirmed when, after the second peak had decayed the addition of glucose gave no response, but the replacement of the ferricyanide solution restored the current to its original maximum value. Thus the increased concentration of potassium ferricyanide allowed for only slightly higher levels of current and did not produce the expected increase in output stability (see Section 4.3). The replacement of the cathodic solution was not a practical method for maintaining current levels, therefore possible alternative cathodic reactants were investigated.

3.6 Alternative Cathodic Reactants

3.6.1 Oxidised HEF

HEF had been shown to be highly electroactive at the carbon cloth electrodes, and extremely stable. In the anodic compartment the HEF was recycled between the enzyme and the electrode, hence only a 2.5mM concentration was required. In the cathodic compartment, on the other hand, the HEF would not be recycled, hence a higher concentration was required and this raised the problem of solubility.

A range of solvents were screened for their suitability before 50% dimethylformamide (DMF)/50% KH_2PO_4 buffer pH6.0, 40mM HEF was chosen as the cathodic electrolyte. Before use in the fuel cell the HEF had to be oxidised. This was accomplished by placing the electrolyte in the working compartment of the potentiostat (with 9ml solvent in the counter compartment) and holding the potential at +280mV for 3 hours - at this time the absorbance of the solution at 630nm (λmax for HEFox) ceased to increase any further. The electrolyte was then placed in the cathodic compartment of the fuel cell. The anodic compartment contained 9ml electrolyte-0.25M sodium carbonate buffer pH10.0, 2.5mM HEF, 0.4mg GOD and 50mM glucose.

A maximum current of 860μA was produced (stable at maximum for 2½ hours), with a $t_{½}$ of 5 hours. The oxidised HEF was totally exhausted after 7 hours, but the fact that the cell had maintained a stable maximum

current was encouraging. Increasing the concentration of the HEF was not possible due to the problem of solubility; therefore an attempt was made to chemically recycle the HEF in the cathodic compartment.

3.6.2 HEF/Potassium Dichromate

In order to try to chemically recycle HEF in the cathodic compartment a number of compounds were tested for their ability to oxidise HEF to its ferricinium ion. The most effective chemical oxidation of HEF was obtained by adding 50mM $K_2Cr_2O_7$ to the cathodic electrolyte- 9ml 50% DMF/50% sodium acetate buffer pH2.0, 40mM HEF- before use in the fuel cell. This brought about the complete oxidation of the HEF and raised the pH of the solution to 5.5. The anodic compartment was set up as in 3.6.1. The performance of the configuration is shown in Table 3.13.

Table 3.13 Response of the cell to additions of glucose, chromate and HCl See text for experimental conditions.

Addition	Maximum Current(mA)	$t_{½}$(hr)	pH at end of peak Anode	Cathode
Glucose	0.98	18	9.7	7.5
Glucose/$K_2Cr_2O_7$	0.58	-	-	-
HCl	1.06	12	9.3	6.7
HCl	1.00	13	8.7	6.8

After the first addition of glucose the current was stable

at the maximum for 4 hours before gradually decaying, which was an improvement on the previous system. The increased stability suggested that some recycling of the ferrocene may have occured. The second addition of glucose had no effect on the decayed current, and the addition of $K_2Cr_2O_7$ to the cathodic compartment only gave a slight response until the pH was lowered to 4.5. The peaks produced by the reduction of pH began a gradual decay immediately suggesting that (i) the HEF may have only been partly reoxidised and (ii) the $K_2Cr_2O_7$ was also reduced at the cathode.

A control experiment was therefore set up to determine whether or not $K_2Cr_2O_7$ was reduced. An identical configuration to the above was used, but with the HEF omitted. A maximum current of 800μA was produced with a $t_{\frac{1}{2}}$ = 3 hours. The reduction of the cathodic pH produced another current peak of similar stability. To confirm that the observed current was due to the reduction of $K_2Cr_2O_7$ and not the reduction of any oxygen in the cathodic compartment the experiment was repeated with oxygen-free nitrogen gassed through the compartment. A maximum current of 860μA was produced with identical stability and pH sensitivity.

The current was thus due solely to $K_2Cr_2O_7$ reduction:-

$$7H^+ + Cr_2O_7^{2-} + 6e^- \longrightarrow 2Cr^{3+} + 7OH^-$$

which explains its pH sensitivity.

The initial current produced in the configuration containing both HEF and $K_2Cr_2O_7$ in the cathodic compartment

would then be due to a combined reduction of the two species. The current produced by lowering the pH was most probably due to the following:-

$$7H^+ + Cr_2O_7^{2-} + 6Fc^{2+} \xrightarrow{+6e^-} 2Cr^{3+} + 6Fc^{3+} + 7OH^-$$

and direct dichromate reduction at the cathode.

HEF was unsuitable for use as the reactant at the cathode due to its poor solubility, and the attempt at recycling, while improving the stability, introduced again the problem of pH control.

3.6.3 Methylene Blue (MB) and Methyl Viologen (MV)

The above mediators were not as electroactive as HEF at the carbon cloth electrodes (Table 3.2), but as they are readily oxidised by oxygen, it was hoped that they could be recycled producing a stable cathodic reaction.

The anodic compartment was set up as in 3.6.1. The cathodic compartment contained 9ml electrolyte - 0.25M KH_2PO_4 buffer, pH6.0, 2.5mM mediator and was initially gassed with oxygen-free nitrogen to assess cell performance without any recycling. Table 3.14 shows the response of the cell before and after oxygen was added to the cathode.

The initial currents were not encouraging and had very poor stability. Changing the gassing from nitrogen to oxygen had no effect on the current until the pH of the cathodic compartment was lowered to 2.0 (as in section 3.4.1). The resultant output was then more

consistent with the pH dependent reduction of oxygen at the cathode than with recycling of the mediator. Either way, methylene blue and methyl viologen did not appear suitable for use as cathodic reactant; and it was clear that substantial further experimentation would be required to optimise the cathodic reaction.

Table 3.14 Performance of the cell employing MB or MV as the cathodic reactant.

See text for experimental conditions.

	Maximum current(μA)		$t_{½}$(hr.min)	
Addition	MB	MV	MB	MV
Glucose/N_2	360	245	2.10	1.05
0_2/HCl	860	500	8.45	9.50
HCl	720	440	9.50	11.00

3.7 Factors Governing Fuel Cell Performance

Once a successful configuration had been achieved with the GOD fuel cell, most of the subsequent experimentation concentrated on the cathodic reaction. To complete the analysis of the fuel cell's performance the factors effecting the anodic reaction (i.e. the enzyme, substrate and mediator concentrations) and the effect of the external resistance (R_{ex}) were examined.

3.7.1 Glucose Concentration

The anodic compartment contained 9ml electrolyte-0.25M sodium carbonate buffer, pH10.0; 2.5mM HEF, 0.4mg GOD and the glucose concentration was varied between 0.75mM and 50mM in seven separate runs. The cathodic compartment contained 9ml electrolyte - 0.25M KH_2PO_4 buffer pH6.0, 250mM potassium ferricyanide, and was gassed with oxygen-free nitrogen. Table 3.15 and Figure 3.9 show the effect of varying the glucose concentration on current output.

The Km of the enzyme was found from spectrophotometric assay to be 1.3mM at pH10.0.
N.B. The possibility of product inhibition was eliminated by the following control - the experiment with the glucose concentration at 25mM was repeated, with 25mM gluconic acid added to the anodic compartment; the maximum current produced was 1.04mA with a t3/4 of 9 hours.

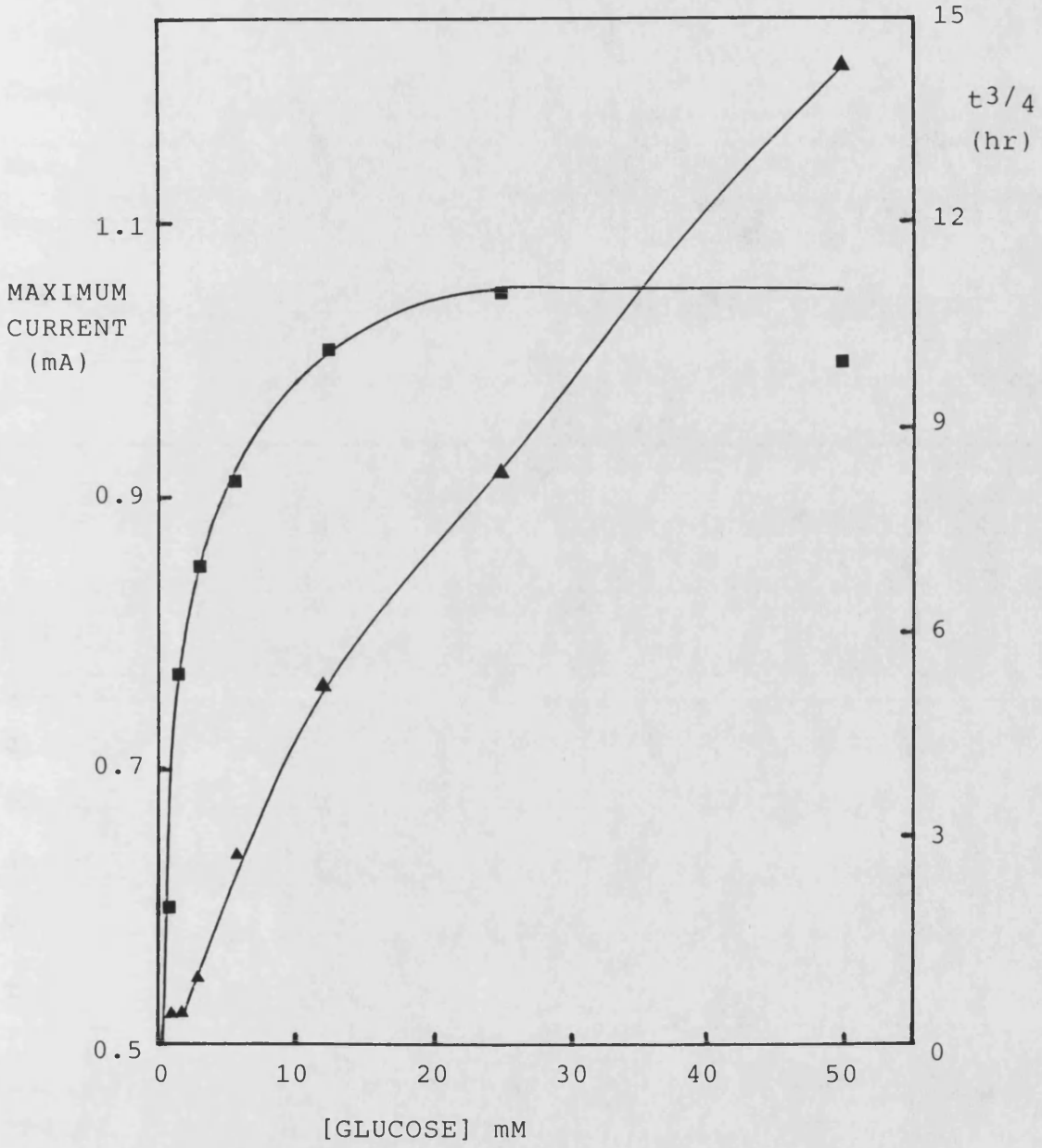


FIGURE 3.9. EFFECT OF GLUCOSE CONCENTRATION ON CURRENT OUTPUT. MAXIMUM CURRENT:- ■——■; t3/4:- ▲——▲.

(see text for experimental conditions)

Table 3.15 Effect of glucose concentration on the current output See text for experimental conditions

Glucose Concentration(mM)	0.75	1.50	3.00	6.00	12.5	25.0	50.0
Maximum Current(mA)	0.60	0.77	0.85	0.91	1.01	1.05	0.98
t3/4 (hr.min)	0.30	0.30	1.00	2.45	5.15	8.30	14.30

3.7.2 Enzyme Concentration

The anodic compartment contained 9ml electrolyte-0.25M sodium carbonate buffer, pH10.0, 2.5mM HEF, 25mM glucose and the amount of enzyme was varied between 0.0032mg and 2.00mg in five separate cell runs. The cathodic compartment was set up as in 3.7.1. Table 3.16 and Figure 3.10 show the effect of varying GOD concentration on current output.

Table 3.16 Effect of enzyme concentration on current output See text for experimental conditions.

Amount of enzyme (mg)	0.0032	0.016	0.080	0.40	2.00
Maximum Current(mA)	0.40	0.80	0.92	1.05	1.15
t3/4 (hr.min)	7.30	8.30	8.00	8.30	8.30
Current Efficiency(%)	133	53.0	11.9	2.7	0.6

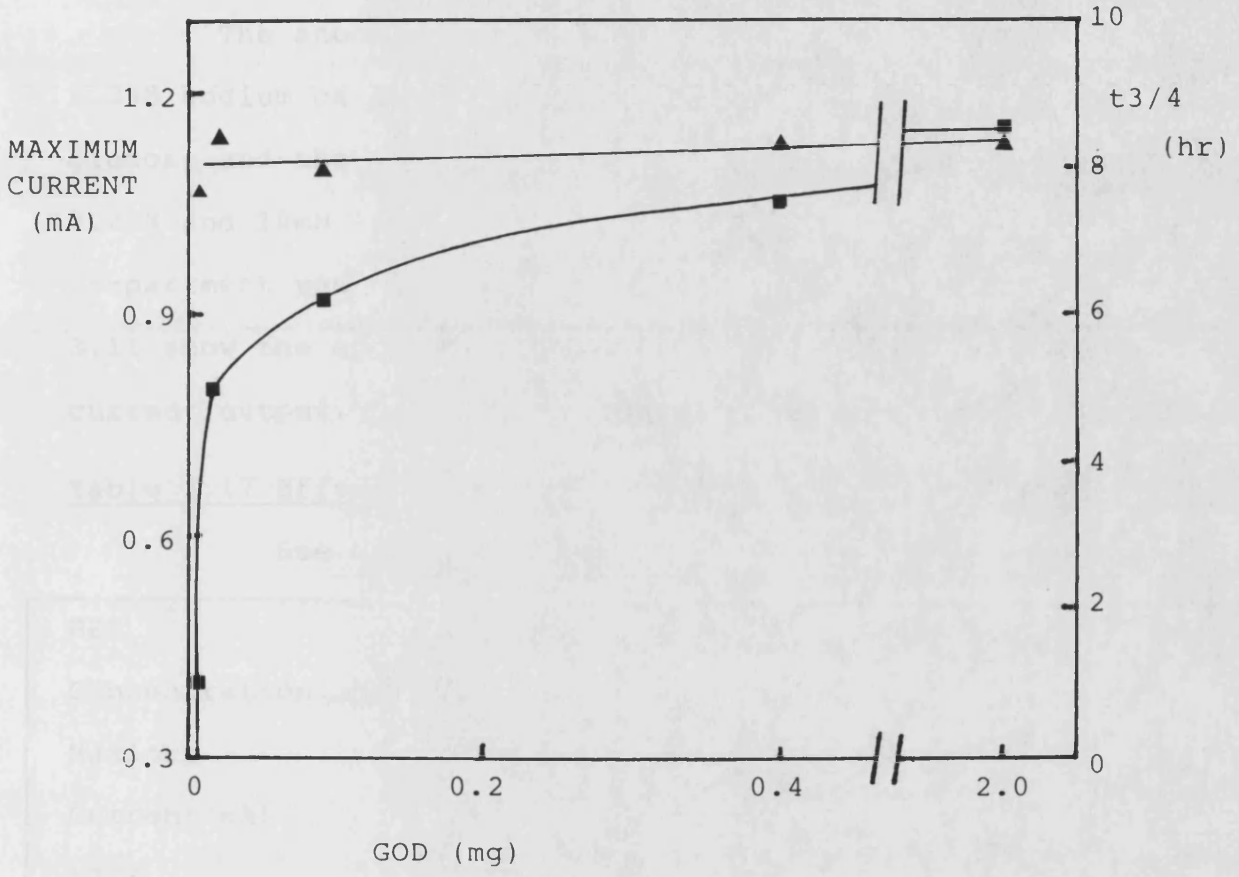


FIGURE 3.10. EFFECT OF GOD CONCENTRATION ON CURRENT OUTPUT

MAXIMUM CURRENT:- ■——■; t3/4:- ▲——▲.

(see text for experimental conditions)

The high current efficiency value obtained with 0.0032mg of enzyme was due to the background current which became significant only at very low current densities.

3.7.3 HEF Concentration

The anodic compartment contained 9ml electrolyte-0.25M sodium carbonate buffer, pH10.0, 0.4mg GOD, 25mM glucose and the concentration of HEF varied between 0.25M and 10mM in five separate cell runs. The cathodic compartment was set up as in 3.7.1. Table 3.17 and Figure 3.11 show the effect of varying HEF concentration on current output.

Table 3.17 Effect of HEF concentration on current output

See text for experimental conditions.

HEF Concentration(mM)	0.25	0.50	1.00	2.50	10.0
Maximum Current(mA)	0.49	0.79	1.00	1.05	1.10
t3/4 (hr.min)	8.00	8.30	7.30	8.30	9.00

3.7.4 External Resistance

The anodic compartment contained 9ml electrolyte-0.25M sodium carbonate buffer, pH10.0, 2.5mM HEF, 0.4mg GOD and 25mM glucose. The anodic compartment was set up as in 3.7.1, and the external resistance was varied between 2Ω and 1000Ω in five separate cell runs. Table 3.18 and Figure 3.12 show the effect of varying the

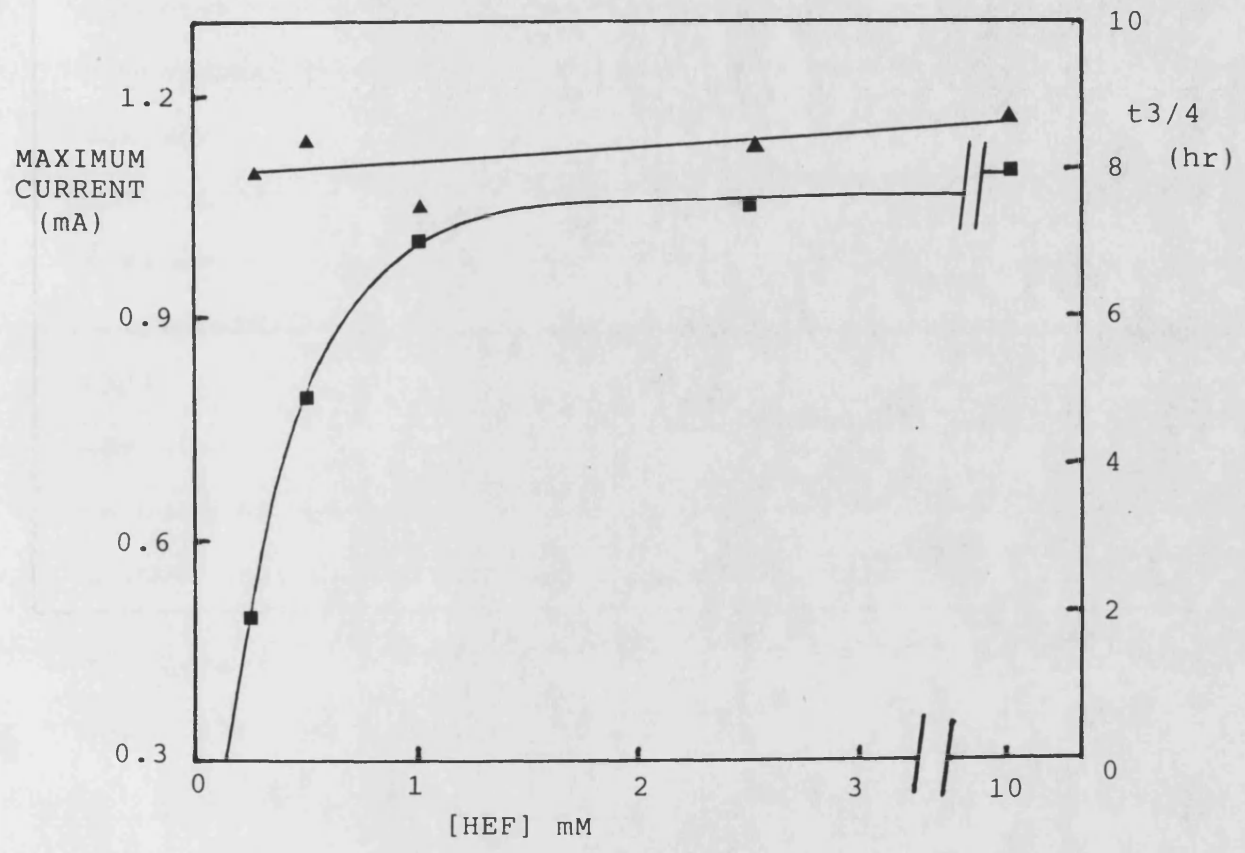


FIGURE 3.11. EFFECT OF HEF CONCENTRATION ON CURRENT OUTPUT

MAXIMUM CURRENT:- ■——■; t3/4:- ▲——▲.

(see text for experimental conditions)

external resistance on the output of the cell.

Table 3.18 Effect of external resistance on cell output

See text for experimental conditions

External Resistance(Ω)	2	10	50	250	1000
Maximum Current(mA)	1.04	1.05	0.88	0.53	0.21
Maximum Voltage(mV)	207	221	228	236	266
t3/4 (hr.min)	8.00	8.30	10.30	17.00	32.00
Calculated open * circuit voltage(mV)	294	309	301	281	283

* Values for the open circuit voltage of the cell were calculated using the equation:-

$$E = V + IR_{INT}$$

The internal resistance was taken as 84Ω (Section 3.4.2).

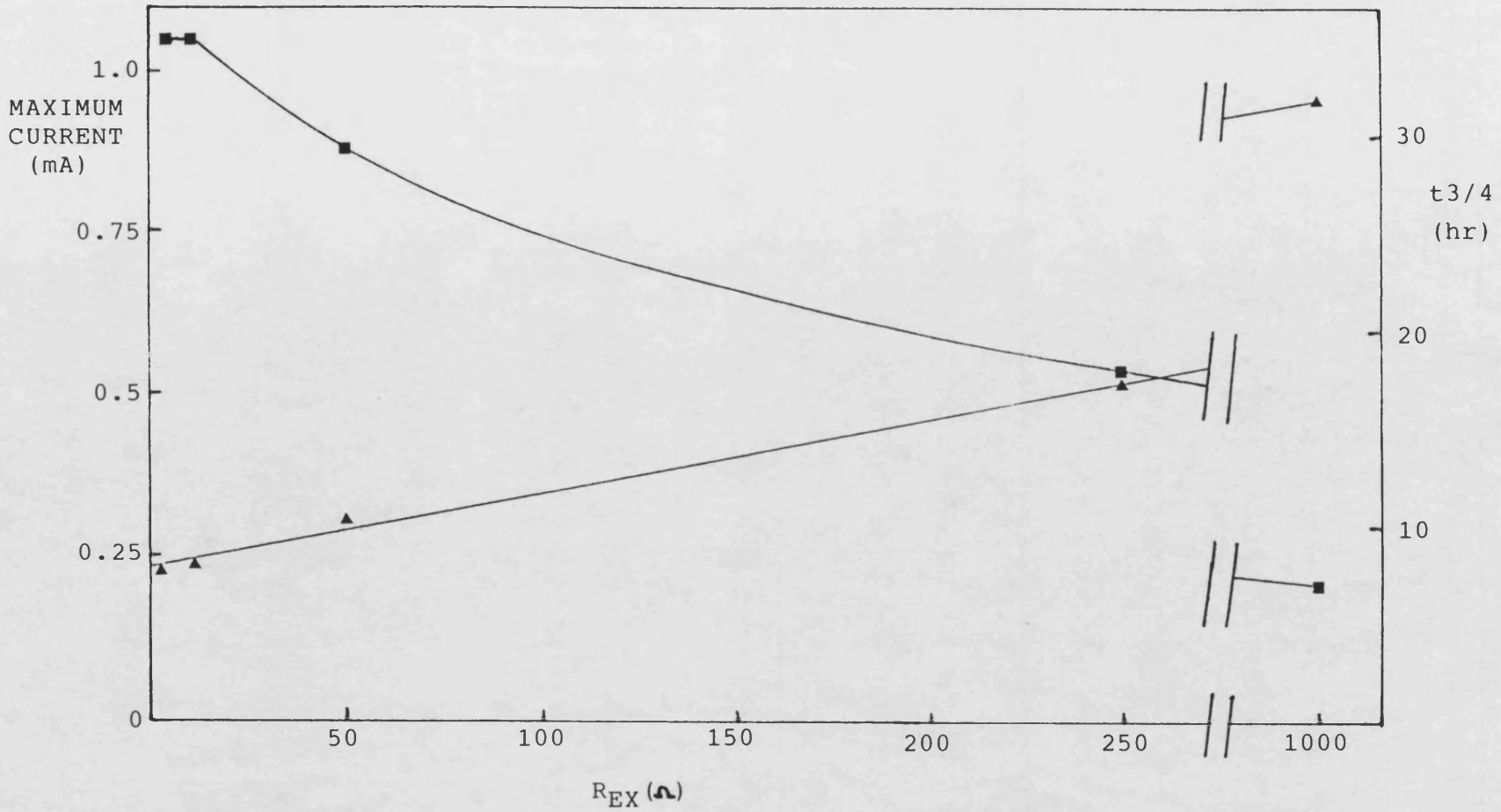


FIGURE 3.12. EFFECT OF EXTERNAL RESISTANCE ON CURRENT OUTPUT.

MAXIMUM CURRENT:-■——■; t3/4:-▲——▲.

(see text for experimental conditions)

4 DISCUSSION

The development of the fuel cell was, on the whole, concerned with increasing the stability of the output. Once a comparatively stable output was achieved the performance of the cell was evaluated more fully. The initial difficulties with stability were mainly due to the choice of enzyme (Section 3.1); hence no real progress was made until a more suitable enzyme was employed; but some novel applications of enzyme immobilisation were attempted. The choice and performance of enzymes in the fuel cell will be discussed in Section 4.1.

A range of mediators was screened and the most promising candidates chosen for use with glucose oxidase in the fuel cell (Section 3.2.1). The factors governing the suitability of mediators for fuel cell applications will be discussed in Section 4.2. Enzyme immobilisation was not found to increase the stability of the fuel cell output, and the replacement of the carbon cloth by platinum gauze electrodes gave a reduced performance from the cell. The experiments with the potentiostat led to the investigation of the effects of the pH and the cathodic reaction on the stability of the fuel cell output. These two factors were found to be crucial to the development of a stable system and will be discussed in Section 4.3. Once a relatively stable system had been developed the physical design of the cell was altered to attempt to improve the performance characteristics. The restrictions imposed by the various aspects of cell design will be discussed in Section 4.4.

4.1 Enzyme Suitability

(i) Alcohol oxidase

The initial experiments using alcohol oxidase with PES as the mediator (Section 3.1.1) gave very disappointing results - the poor stability was thought to be due to denaturation/deactivation of the enzyme. The experiments under potentiostatic control (Section 3.1.6) showed that the current produced by the alcohol oxidase system were due to a reaction between the enzyme and the mediators. The lack of a response to methanol could be explained by such a reaction resulting in the formation of an inactive complex. Once aliquots of the reaction mixture were removed from the potentiostat for assay (a 60 fold dilution) over 60% of the original activity was found to be present, hence it is possible that the complex could have dissociated on dilution. Due to its reactivity with mediators alcohol oxidase was not considered further as a catalyst for the development of the biofuel cell.

(ii) Yeast alcohol dehydrogenase

In comparison with alcohol oxidase, YADH is cheaper and has a much higher specific activity; however, an expensive cofactor is required. Therefore the enzyme is not an ideal choice for a biofuel cell. The system was useful, though, in assesing the suitability of various immobilisation procedures.

YADH did not react with the mediators as had

alcohol oxidase, but the cell still produced currents with very poor stability (Section 3.1.2). The loss of current was seen to be mirrored by a loss of enzymic activity (Figure 3.2), and enzyme immobilisation appeared a promising approach to reduce these losses. Simple physical adsorption on the electrode surface did not produce any significant stabilisation, while the entrapment of the enzyme within polymeric gels set around the electrode reduced the current output, but increased the stability. Unfortunately, a gel that was both flexible and strong enough to withstand the compartmental conditions could not be found. More importantly, it appeared that the enzyme only had a limited stability in the vicinity of the elctrode. The specific mechanism of the denaturation process would have been extremely difficult to determine, so immobilisation away from the electrode was attempted.

The optimum performance for a YADH biofuel cell was achieved using the enzyme immobilised in agarose beads, but again there was an almost total loss of activity by the end of a cell run (Section 3.1.5). This was probably due to the accumulation of the beads around the carbon cloth during the run. In this specific system the beaded enzyme was not particularly successful, but the use of beaded enzymes in fuel cells generally, could be extremely advantageous. The enzyme, due to its immobilisation, would have increased stability, and could be recovered easily by centrifugation but unlike most other forms of immobilisation could move freely throughout the fuel cell compartment reducing diffusional

effects on reaction rates.

Poor diffusion was thought to be responsible for the low current produced when the enzyme was immobilised in carrageenan gel next to the cation exchange membrane, and the loss of stability meant that this approach was not given further consideration in the development of the biofuel cell. Immobilisation of the enzyme to nylon mesh also proved unsuccessful and it was concluded that YADH was unsuitable for use in a biofuel cell due to its inherent instability in the anodic compartment.

At present the most suitable enzyme for alcohol oxidation in a biofuel cell is a quinoprotein alcohol dehydrogenase isolated from methylotrophic bacteria (Davis et al., 1983). However, the enzyme has a relatively low unit activity and requires an extensive isolation procedure, so it was not chosen for this study.

(iii) Glucose oxidase

In comparison with the above enzyme GOD appears a much more suitable candidate for use in the development of a biofuel cell. The enzyme is comparitively cheap, readily available, has a high specific activity and has previously been employed at a biofuel cell anode (e.g. Weibel and Dodge, 1975; Laane et al., 1984a, b). GOD did not undergo a substrate independent reaction with the mediators as had alcohol oxidase, and was extremely stable in the fuel cell, in marked contrast to YADH. With the GOD system the stability was mainly dependent on the mediator, pH and the cathodic reaction. Under potentiostatic control (Section 3.3) the system consistently gave

coulombic efficiencies greater than 90%, and under enzyme limiting conditions the biofuel cell showed excellent current efficiency (Section 3.7.2).

In the early stages of the development of the GOD fuel cell (Section 3.2.2) the enzyme was successfully immobilised to a nylon membrane and in a protein film, without any severe limitation on the current output as had occurred with the YADH immobilisation attempts. This suggested that, if required, the system could be developed to function just as effectively with immobilised enzyme.

Another possible advantage of the glucose oxidase fuel cell is that it could be adapted to run off a range of fuels. The addition of cellulases, invertase and mutarotase, or α-amylase and amyloglucosidase could allow the production of electricity (and chemicals) from cellulose, sucrose or starch (Laane et al., 1984a).

4.2 Mediator Suitability

So far, in the development of enzyme biofuel cells, higher current densities have only been achieved by the employment of low molecular weight diffusable mediators to facilitate electron transfer from the enzyme redox centre to the electrode (Higgins and Hill, 1985). This approach while being far from ideal, as discussed in Section 1.3.3, was therefore used in the development of the fuel cell.

The original choice of mediator, PES, appeared the most suitable of those used in the YADH system. However, the restrictions imposed on the performance of the cell by the stability of the enzyme meant that the mediator could not be assessed for long term use with this system. Turner and Davis (1982) found PES to be unsuitable for long term use due to its gradual decomposition and the deposition of reduced PES on the cell surfaces and the dividing membrane. Such deposits were observed at the end of a cell run with the YADH system (and with GOD). It seems likely, therefore, that even if the problem of enzyme stability had been overcome, the performance of the cell would then have been limited by the behaviour of the PES.

The assessment of mediator for use with GOD was again undertaken directly in the fuel cell as in this way the combined effects of :-

(i) the rate of electron transfer from enzyme to mediator,

(ii) the rate of mediator reoxidation at the electrode,

(iii) the rate of mass transfer of the mediator, and, (iv) the stability of the mediator under fuel cell conditions on the performance of the cell could be ascertained. A detailed examination of some of these processes, e.g., using cyclic voltammetry would have been the ideal approach, but at that stage in the development of the cell, the experiment was only designed as a rapid screening before the immobilised enzyme was employed.

Table 3.2 shows that some of the mediators were more suitable to the GOD/carbon cloth system than others, e.g., PES had produced much higher currents with YADH. Hence, it appeared that the mediator was not capable of rapid electron transfer with GOD. In contrast DCPIP which had been used quite successfully with GOD at platinum electrodes (Laane et al., 1984b) presumably gave a low current because of a slow reaction at the carbon cloth electrode. Indeed, the replacement of the carbon cloth with platinum electrodes (Section 3.2.4) resulted in a higher current with this mediator. Benzoquinone was obviously well suited for electron transfer in this system, producing the highest currents, along with HEF which also yielded a respectable peak current. It is interesting to note that when the system was under potentiostatic control (Section 3.3) HEF gave the higher currents : this may have been due to an increased rate of polymerisation of the benzoquinone in the potentiostated cell. Toluidine Blue-O and Methylene Blue were not as well suited to the GOD/carbon cloth system as benzoquinone and HEF. The reduced rates of electron transport could

have been partly due to an increased sensitivity to oxygen in these anodic systems as it was noted that if the cathodic gassing was stopped there was a temporary current increase, due to reduced oxygen diffusion through the cation-exchange membrane (see Section 1.3.3).

The variation in the stability of the current output with the differing mediators was probably due to a number of factors. It was later shown that the stability of the HEF mediated cell was dependent on the stability of the pH of the compartments (Section 3.5.1) and the cathodic reaction (Section 3.6). It is almost certain that the stability of the cells with other mediators could have been improved by paying attention to these aspects. However, due to their reduced stability in this experiment, it is likely that such improvements would have been limited. The mediator-related factors thought to be responsible for the reduced stability are:-

(i) the instability of the mediator itself, i.e., decomposition or polymerisation may occur,

(ii) adsorption of the mediator or derivatives of the mediator on the cation-exchange membrane and/or the electrode,

(iii) an inactivation reaction with the enzyme.

It is quite possible that for a given mediator a combination of these factors was responsible for the current decay, but even without a detailed analysis, certain tentative conclusions could still be made. The instability of PES had already been discussed. DCPIP appeared to be unstable, as a temporary reversal in current decay could be achieved by adding further amount of the mediator. Adsorption of the mediators by the cation-

exchange membrane could be observed by a discolouration of the membrane. The extent of the absorption could not be easily assessed quantitatively, and it was found that the re-use of membranes with benzoquinone and HEF had no effect on the performance of the system. Hence the stability was not significantly limited by membrane adsorption in these cases. Any effect of the adsorption of the mediator by the electrode was also difficult to assess. Benzoquinone preadsorbed onto the cloth gave similar results to the mediator in free solution, indicating that absorption was not responsible for current decay. However, it is likely that the pattern of adsorption would be different on the charged electrode in the fuel cell; hence the effect of adsorption could not be totally discounted.

Complex formation between GOD and the mediators could have led to different rates of inactivation of the enzyme. This inactivation may in some cases have been reversible, i.e., once a sample of the anodic solution was added to the assay mixture (a 30 fold dilution) the complex dissociated to give active enzyme, e.g., on incubation with benzoquinone there appeared to be no loss of activity, and in the cell the loss of activity in solution did not correlate with the current decay (Section 3.2.3). One possible explanation is that the majority of the enzyme was absorbed by the electrode and then both the free and absorbed enzyme gradually formed inactive complexes with the mediator.

HEF was finally chosen as the most suitable mediator for the development of the GOD biofuel cell as

it (i) was extremely stable in the fuel cell, even in aerobic conditions, (ii) gave rapid electron transfer, and (iii) did not inhibit or interact with the enzyme.

4.3 The Cathodic Reaction and the Effect of pH

In virtually all reported enzyme fuel cells the cathodic reaction is assumed to be the reduction of oxygen to water. The reaction is slow and often inefficient, hence it is likely to be a limiting factor on the perfromance of a fuel cell (Wingard et al., 1982). In addition, the fact that a low pH was required to drive the reaction (van Dijk et al., Section 3.4.1) meant that the stability of the current output was adversely effected. This occured because the pH in the cathodic compartment was continually rising due to (i) equilibration with the anodic electrolyte (pH8.0) across the cation exchange membrane and (ii) the consumption of hydrogen ions and the possible production of hydroxide ions by the cathodic reaction. The equilibration of the buffers across the membrane also resulted in a reduction of the anodic pH below the optimum range (pH8-10 - see Section 3.5) which contributed to the current decay.

In summary, the reduction of oxygen was not found to be a suitable cathodic reaction for the developmental GOD fuel cell. Reasonable current levels (approaching 1mA) could be obtained only at the expense of the output stability. Alternative cathodic reactions were, therefore, investigated.

Workers studying microbial fuel cells had found that potassium ferricyanide reduction gave a stable and relatively nonpolarisable cathodic reaction (Allen, 1972; Bennetto et al., 1983). This reaction was the

most successful of those employed at the cathode of the developmental fuel cell, and was found to be pH independent (Table 3.11). The stability of the current output was seen to be limited solely by the cathodic reaction (provided that the glucose concentration was kept high in the anode) e.g., in Section 3.5.2 once a current peak had decayed, the replacement of the ferricyanide solution was sufficient to restore the maximum current level.

It appeared that there was some limitation on the efficiency of the ferricyanide reduction, though, as rough estimations of the number of coulombs passed during the current peaks indicated that the supply of the cathodic reactant should not have been exhausted. The use of 1M ferricyanide (Section 3.5.3) supported this view as the increase in reactant concentration did not result in a proportional increase in the stability of the output. In addition the current again began to decay as soon as the maximum value had been attained. With oxygen as the cathodic electrolyte the output was clearly limited by changes in pH; with ferricyanide the reason for the limited stability was not apparent, but it is possible that the reaction was not as free from polarisation effects as was first thought.

In contrast the reduction of the HEF at the cathode appeared to be a stable and efficient process (Section 3.6.1). Unfortunately, if a cell were to be run for any length of time, frequent changes of the cathodic electrolyte (or perhaps a continuous flow of

electrolyte through the compartment) would be required due to the poor solubility of the ferrocene. Chemical reoxidation of HEF in the cathodic compartment did not appear a feasible alternative mainly due to the low pH requirement. A possible alternative would to be to couple a reductase enzyme to this cathodic reaction, but the chance of obtaining an enzyme that would oxidise HEF as rapidly and efficiently as GOD reduces HEF could be very slight. A more feasible alternative would be to screen a range of ferrocenes to find one that was both highly soluble and easily reduced at the cathode.

Methylene blue and methyl viologen were not found to be suitable cathodic reactants and the attempt to recycle the mediators by the addition of oxygen merely resulted in the cathodic reaction reverting to the pH dependent reduction of oxygen.

The brief investigation of alternative cathodic reactants indicated that there is a wide scope for further work on this aspect of biofuel cells. The most promising direction for such work would be to attempt to obtain an efficient, stable, recyclabe enzyme-mediator system, that would in effect be operating in reverse to the anodic system. Until such a system could be developed a full screening of alternative electron acceptors might yield a more suitable reaction than the reduction of ferricyanide.

4.4 Cell Design

Many types of cell have been used in bioelectrochemical studies, the choice of design depending on the particular aims of the work. For Galvanic studies such as those used with the developmental fuel cell one of the main requirements is a good conducting pathway through the cell, which is also capable of chemically isolating one half-cell from the other (Allen, 1972). Salt-agar bridges have been used for this purpose (Weibel and Dodge, 1975; Laane *et al.*, 1984b) but their relatively high resistance is a disadvantage. A more popular method has been the use of cation-exchange membranes which are excellent separators as they are essentially impervious to the transport of water or other uncharged particles (Allen, 1972; Turner *et al.*, 1982) and have a low electrical resistance (see Appendix I).

The use of a cation-exchange membrane meant that the design of the cell had to allow for an efficient method of sealing the membrane between the compartments without causing lengthy assembly and cleaning procedures. The design employed for the developmental fuel cell (Figure 2.1) met these requirements; there were very few leakage problems, and the cell could be assembled in a few minutes.

It was thought that the shape of the compartments housing the bulky carbon cloth electrodes led to inefficient mixing and hence to the formation of 'dead zones' which might have limited the performance of the cell. However,

when the design was altered with the cloths looped inside the perimeter of the compartments (Figure 3.8) to allow more efficient mixing of both the bulk solution and at the electrode and membrane surfaces, there was no improvement in performance. Indeed, the removal of the electrodes from the proximity of the membrane resulted in a reduced performance due to increased ohmic polarisation (Section 3.4.3).

Future cell designs should ,therefore, have both the electrodes positioned as close to the membrane as is practically possible. In addition increasing the ratio of the membrane area to the compartmental volume may also improve performance by reducing internal resistance. For systems where the cathodic reaction may be limiting the output of a cell (Section 4.5) it would also be more efficient to have an increased cathodic area. A basic design fulfilling the above requirements is shown in Figure 4.1. Unfortunately, the BDH cation exchange membrane was found to be too inflexible for such a design and substantial leakage problems were encountered. The assessment of the effectiveness of the design therefore awaits the development of a more flexible cation exchange membrane.

Looking further ahead, if a relatively high output, long term cell was developed the next logical step would be to create a battery of cells to obtain a more practically useful output level (Turner et al., 1982). The construction of a battery would clearly introduce further constraints on the cell design, similar to some of those encountered with conventional fuel

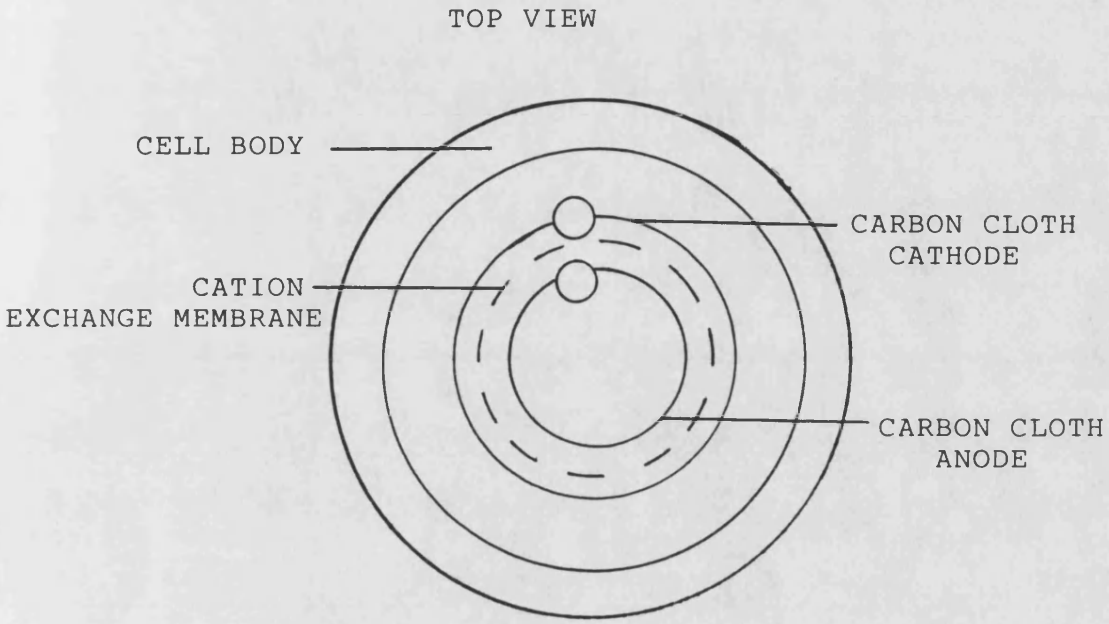


FIGURE 4.1. DESIGN PROPOSAL FOR IMPROVED PERFORMANCE

(Drawn to scale)

cells e.g. all the cells in the battery must:-

(i) be suitably interconnected electrically,

(ii) receive adequate supplies of fuel and oxidant,

(iii) remain free from leaks, and

(iv) have a low internal resistance (Pearson, 1966).

4.5 Final Comments

The investigation of the relationships between the concentration of the anodic constituents and the current output, and the external resistance and the current output, gave further indications as to the importance of the cathodic reaction and the design of the cell.

The maximum current initially increased with increasing glucose concentration, but at the highest concentrations the maximum current became essentially independent of the substrate concentration (Table 3.15 and Figure 3.10). Such saturation with the substrate is a characteristic of all enzyme catalysed reactions. However, if the kinetics of the reaction are considered then it appears that other factors have limited the maximum current. The Km of the enzyme was found to be 1.3mM at pH10.0. Figure 3.10 shows that a current of around 0.75mA would be produced at this concentration. Therefore, at saturating glucose concentration a maximum current of around 1.5mA could ideally be expected under these experimental conditions.

The stability of the current output obviously increased with increasing glucose concentration, but the increase was not linear as the cathodic reaction became more of a limiting factor with increasing peak length.

Figure 3.11 and Table 3.16 show that initially the maximum current was extremely sensitive to the enzyme

concentration, correlating with the high current efficiency; but with increased enzyme concentration the maximum current obtained only increased relatively slightly, with a corresponding decrease in efficiency. In addition the stability of the current output was shown to be virtually independent of the enzyme concentration.

Similar results were obtained in the study of the HEF concentration - Table 3.17 and Figure 3.12. At lower (<0.75mM) HEF concentrations the maximum current was almost proportional to the HEF concentration. Raising the concentration above 1mM resulted in only slight increases in the current, which was again limited around 1.1mA. The stability of the current output was also virtually independent of the HEF concentration.

Davis et al.(1983) achieved similar results in their concentration studies and suggested that the maximum current was limited by the rate of mass transport of the reduced mediator. This was also partly the case with the developmental glucose fuel cell as the current was found to increase slightly with increased rate of stirring. The maximum current has also been seen to be influenced by the choice of mediator and the cathodic reaction. The influence of the cathodic reaction on the output of the cell has been quite marked and indicates that in the concentration studies it was probably the major current limiting factor. It is clear, though, that for any one system a number of factors and the interactions between these factors combine to limit the maximum current. A systematic investigation employing

a factorial series of experiments would,therefore,be required to gain a full understanding of the roles of such factors as the cathodic reaction, rate of electron transfer from enzyme to mediator, rate of mass transport of the mediator, rate of electron transfer from mediator to electrode and cell design in current limitation. The attribution of the rate of mass transport as the sole factor responsible for current limitation by Davis et al. (1983) thus seems oversimplified. It must be said that the discussion of the performance of this and other reported fuel cells (e.g. Laane et al., 1984) has generally been far too brief.

The concentration studies, while not explaining the limiting current, did confirm the earlier conclusion (Section 4.3) that the two factors effecting the stability of the output were the level of glucose and the cathodic reaction.

The variation of the external resistance also showed the current to be limited around 1.1mA. Table 3.18 and Figure 3.13 show that at resistances above 10Ω there was an almost linear decrease in the maximum current, but below 10Ω the current was limited by other factors (see above) including the internal resistance of the cell (taken as 84Ω). It is clear that with such a high internal resistance variation in low values of external resistance would have little effect on the current output of the cell.

The stability of the current output increased linearly with increasing external resistance as the

fuel cell was being discharged at a slower rate. The calculated open circuit voltage values were lower than those obtained by direct measurement (Section 3.4.3) as only the effect of ohmic overpotentials was taken into account.

As previously stated (Section 3.4.3) the overall performance of the developmental fuel cell was quite respectable in comparison with other enzyme fuel cells, e.g.,the alcohol dehydrogenase (ADH) cell developed by Davis et al. (1983) which had a maximum current density of $0.2Am^{-2}$, and a maximum power of 12μW, with a power density of $20mWm^{-2}$. The developmental cell gave $1.0Am^{-2}$, 220μW, and $176mWm^{-2}$ respectively. The stability of the ADH cell was higher than that achieved with the developmental cell but it is hoped that this could be reversed with further improvements to the cathodic reaction.

The carbon cloth electrode was shown to be a suitable,low cost alternative to the more conventional electrodes,e.g.,platinum or gold, used in fuel cells. The large effective surface area of the cloth makes it ideal for the production of high current densities, and with improvement in electrode and cell design it is possible that substantial increases in the power output of the cell could be achieved. Even with such improvements it is unlikely that the developmental cell would ever match the performance of the conventional fuel cells,e.g., a Bacon cell at a current density of $215mAcm^{-2}$ and a potential of 0.95V produces a power

density of 2.02kWm^{-2} (McDougall, 1976).

The general shift of research from biofuel cells to biosensors in recent years, particularly with enzyme-based systems, also means that the commercial application of enzyme fuel cells, even in specific low power devices is still some way off. The poor outlook for these devices as power producers, though, is balanced by the exciting application of bioelectrocatalysis in biosensors, and the possible use of biofuel cells, similar to the developmental cell, in the production of (bio)chemicals from a range of substrates (Laane et al., 1984b).

APPENDIX I

Ion exchange membranes

Ion exchange (or, more accurately, ion transfer) membranes may be regarded as ion exchange resins produced in sheets rather than in the conventional form of beads or granules, the essential structure in each case being polystyrene cross-linked with divinylbenzene.

Membranes are made 'cation permeable' or 'anion permeable' by chemical treatment, whereby sulphonic acid ($-SO_3^-$) or quaternary ammonium ($-NR_3^-$) groups respectively are attached to the membranes. The resulting selectivity of the membrane arises from its ion exchange characteristics, e.g. a cation membrane with active $-SO_3H$ groups will have mobile H^+ ions in a negatively charged matrix, so that anions will be rejected by the negatively charged background. Thus cation membranes are permeable only to cations and anion membranes permeable only to anions. This 'permselectivity' is the basis of ion permeable membrane processes.

Properties of cation permeable membrane

Thickness, mm	0.15-0.17
Bursting strength, Kg/cm²	3-4
Resistance Ohms/cm²	2.7-3.2
Transport No.	
Na^+, K^+	0.70
Cu^{2+}, Mg^{2+}	0.28
Cl, SO_4	0.02
Chemical form	Na

Resistance to chemical attack: the membrane is stable in alkalis and acids and in inorganic salt solutions. The resistance to reducing agents is good but prolonged contact with oxidising agents will result in some deterioration in properties.

High thermal resistance: generally suitable for temperatures up to 60°C, but for continuous use a maximum working temperature of 40°C is recommended.

APPENDIX II

Properties of carbon cloth, grade CSA

Carbon cloth CSA is a soft, supple fabric with a good 'Drape' to lay-up on complex curved surfaces. It is heat stabilised to 1260°C and manufactured from continuous filament rayon precursor and has a 8-harness satin weave. It is resistant to flame, penetration by molten metal, and all corrosive chemicals except strong oxidising agents such as concentrated nitric acid. It is electrically conductive and has the general chemical and physical properties of bulk carbon.

Typical Properties

Thickness (mm)	0.5
Weight (g/m^2)	270-290
Breaking strength (kg/cm)	7
Moisture % max.	2
Carbon %	97
Ash %	0.5
Electrical resistance, ohms/square	0.6
Thread count/cm	20

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